USDA/STATE/EPA ASSESSMENT TEAM OF THE
NATIONAL AGRICULTURAL PESTICIDE IMPACT ASSESSMENT PROGRAM
UNITED STATES DEPARTMENT OF AGRICULTURE

USDA/STATE/EPA
ASSESSMENT OF ETHYLENE OXIDE
USES IN AGRICULTURE

COORDINATED BY THE OFFICE OF
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USDA/STATE/EPA ASSESSMENT OF ETHYLENE OXIDE (ETO) USES IN AGRICULTURE

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ABSTRACT

The Environmental Protection Agency (EPA) has issued a notice of rebuttable presumption against registration and continued registration of all pesticide products containing ethylene oxide (ETO). This regulatory action was taken because the EPA concluded that ETO may pose health risks relating to mutagenicity and other chronic or delayed toxic effects, specifically reproductive effects.

More than fifty years ago this chemical was found to be an effective fumigant for insect control. Ethylene oxide is used as a sterilant for foodstuffs, human and veterinary drug products, and medical and laboratory equipment. The commercial production of ETO in the United States was over 5 billion pounds in 1977. The amount of ETO used as fumigants or sterilants is estimated to be less than one percent of total production.

The current U.S. Occupational Safety and Health Administration Standard for exposure to ETO is 50 parts per million (ppm) in air, as a time weighted average concentration for an 8 hour day. There are EPA-established tolerances of 50 ppm on certain stored food products.

Fumigation with ETO is an invaluable tool in the prevention and control of many bee diseases. Contaminated beekeeping equipment is the principal reservoir for bee disease agents. Until ETO fumigation was developed, diseased bee equipment was destroyed by burning. There is no registered alternative material available. Maintaining the beekeeping industry in a healthy condition is essential to our agricultural economy.

Ethylene oxide is used in the many decontamination/sterilization operations that must be followed at the high Containment USDA Research Laboratories to protect laboratory workers and susceptible plant and animal populations. The types of animal and plant diseases which scientists are working with require many safeguards to maximize the containment of the pathogens. There is no known substitute to ETO for many laboratory sterilizations. The loss of ETO as a sterilant and decontaminant in high containment laboratories would seriously compromise biological safety standards or preclude research in these areas of study.

Ethylene oxide is an important chemical recommended by the USDA as a quarantine fumigant against snail infested imported cargo. The loss of ETO would deny the USDA quarantine programs the use of an effective fumigant. The only alternative fumigant is methyl bromide. Methyl bromide cannot be used on all imports because it can cause damage to certain cargo items. Ethylene oxide can be used effectively without damaging the cargo.

Fumigation with ETO is the only means of treating spices and black walnut meats to eliminate pathogens in food prepared and consumed by the general public. There are no approved, effective alternatives to achieve comparable microbial reduction or protection against pathogenic organisms on spices.

The benefits derived from the use of ETO as a fumigant and sterilant and the lack of adequate alternatives show that the chemical is essential to Agriculture and its related industry. Alternative chemicals or other processes have, in themselves, serious limitations or health hazards.

INTRODUCTION

Reason for the Report

The purpose of the report is to evaluate the benefits and the exposure to man, animal, non-target organisms, and the physical environment resulting from registered uses of ethylene oxide (ETO) (1,2-epoxyethane). ETO is used as a fumigant for insects and snails and as a sterilant for many microorganisms. A rebuttable presumption against registration and continued registration of pesticide products containing ETO was issued January 17, 1978 (43 FR 3801).

Background Usage of ETO in Agriculture

More than 50 years ago Cotton and Roark (19) found that ethylene oxide was effective as a fumigant against insects infesting furniture and foodstuffs. Later, Cotton and Young (20) found that added carbon dioxide could increase its insecticidal efficiency. In the next ten years came recognition that ethylene oxide was also effective for destroying bacteria and toward the end of that period patents were granted to Gross and Dixon (32) and to Griffith and Hall (31) concerning the use of ethylene oxide in processes for sterilizing foodstuffs. At that time such ideas apparently attracted little interest, but since then its effectiveness has been amply demonstrated by many investigators and its use in agricultural and industrial fumigation - sterilization processes.

Registered Uses

Ethylene oxide is registered with the EPA as a fumigant and sterilant. There are 38 Federally registered pesticide products

containing ETO as an active ingredient. One Federally registered product contains ETO as an inert ingredient, and there is one application for Federal registration of a State-registered product containing ETO as an active ingredient. In accordance with Section 24(c) of the Federal Insecticide, Fungicide, and Rodenticide Act, three ETO products have been registered in states that have demonstrated that these products are necessary to meet special local needs. ETO is used primarily for sterilization of medical supplies and equipment and as an insecticidual, fungicidal, and bactericidal fumigant on copra, black walnuts, and spices. There are EPA-established tolerances of 50 ppm (40 CFR 180.151) on these stored food products. In addition, ETO is used to disinfect commercial premises, dental instruments, clothing, laboratory animal bedding, laboratory equipment, pharmaceutical equipment, and transportation vehicles, such as jet aircraft, buses, and railroad passenger cars. ETO is also used as a fumigant against certain agricultural related pests.

ETO Production

The production of ethylene oxide (ETO) in the United States in 1977 was reported as 5.28 billion pounds (4). Ethylene oxide is produced by silver-catalyzed oxidation of ethylene, using either air or an oxygen enriched air and is principally consumed in two areas:

1. Ethylene glycol, produced by the hydrolysis of ETO (65% of production).
2. Derivatives, produced by the reaction of ethylene oxide

with various alcohols, ammonia, amines, and organic acids (35% of production).

The market demand for ETO may be segmented into three major categories:

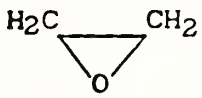
1. For glycols-antifreeze and coolants glycol and fiber-grade ethylene glycol.
2. For derivatives - nonionic surfactants, glycol ethers and ethanolamines. (These derivatives find important uses in synthetic rubber, synthetic fibers, resins, paints, adhesives, plastic film, molded articles, plasticizers, solvents, synthetic detergents, brake fluids, and cosmetics).
3. For all other uses. (Included as other uses would be the usage of ETO as a fumigant or sterilant. Estimated consumption is less than one percent).

PROPERTIES OF ETHYLENE OXIDE

This information has been compiled primarily from the Chemical Safety Data Sheet of the Manufacturing Chemists Association, for Ethylene Oxide, (No. SD-38), 1951, with supplementation from other sources.

A. Alternative Names:

1. Synonyms: 1,2-epoxyethane, oxirane, oxiran, dimethylan oxide, ETO, EO, oxane, dihydrooxirene, oxacyclopropane, oxidoethane, and anprolene.
2. CAS number: 75-21-8.
3. Formula: C_2H_4O



4. Molecular weight: 44.05.

B. Physical/Chemical Properties of ETO

1. Appearance and odor: colorless gas or volatile liquid with a characteristic ether-like odor (irritating in high concentrations).
2. Boiling point: 10.7C (51.3F) at 760 mm Hg.
3. Melting point: -111.30 (-168.3F).
4. Specific gravity: 0.8711 (apparent) (20/20C),(68 F); 0.897 (0/4 C).
5. Vapor density: 1.5 (air = 1).
6. Vapor pressure at 20 C: 1095 mm Hg abs.
7. Solubility: completely miscible with water, alcohol, acetone, benzene, ether, carbon tetrachloride, and most organic solvents. Powerful solvent for fats, oils, greases, waxes, and some rubber formulations.

8. Explosive limits: lower limit 3 percent by volume in air.
Upper limit 100 percent by volume in air.

9. Pertinent chemical properties: highly reactive and flammable; relatively non-corrosive. Reacts with water to produce ethylene glycol, with hydrogen halides to produce ethylene halohydrins, with alcohols and phenols to produce ethylene glycol ethers, with acids to produce ethylene glycol esters, and with amines to produce ethanolamines.

ETO Threshold Limits

The current U. S. Standard (OSHA) for occupational exposure to ETO is 50 parts per million (ppm) parts of air, as a time weighted average (TWA) concentration for an 8-hour exposure (20 CFR 1910.1000), which corresponds approximately to 90 milligrams per cubic meter of air (mg/cu m). The Food and Drug Administration has proposed restrictions on the continued use that would (1) establish maximum residue limits for ETO and its two main reaction products, ethylene chlorohydrin and ethylene glycol, in drug products for human and veterinary use and medical devices, and (2) establish maximum daily exposure levels for drug products for ETO and its two major reaction products (43 FR 27474, June 23, 1978).

Potential Exposure and Hazard

Ethylene oxide must be regarded as poisonous to man by inhalation, although it is not as lethal in comparatively low concentrations as some other fumigants. The threshold limit

value of 50 ppm for continuous daily breathing is higher than that set for many fumigants.

The potential exposure and hazard would be with: (1) individuals who use the pesticide in the operation of ETO sterilizers on a routine basis and/or spend most of their time with operation/aeration procedures, and (2) individuals who apply ETO as a fumigant on a routine basis and/or workers who assist with the fumigation operations.

Exposure potential is a periodic situation in which most of an individual's working day is without potential exposure. The greatest exposure potential may be identified as during the application of the fumigant/sterilant, leakage from the chamber or enclosure, loading and unloading of the treated materials, and during the aeration period. Necessary precautions to avoid employee exposure to ETO can be established or improved.

RPAR Triggers

The rebuttable presumption is based on evidence that ETO causes mutagenic and reproductive effects.

Mutagenic Effects of ETO

> EPA regulations (40 CFR 162.11(a)(3)(ii)(A)) state "that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation products(s) induce mutagenic effects as determined by multitest evidence". The EPA, in its position document, claims that ETO is a general point (gene) mutagen in prokaryotic (bacterial) species) and eukaryotic (animal and higher plant) systems. EPA states that this means that ETO can

interact with DNA of various species to produce mutations in both reproductive and other body cells. The position document indicates there is evidence that ETO can induce chromosomal mutations in somatic cells of humans and other mammals. In addition, it is claimed that ethylene chlorohydrin (ECH), an ETO degration product, acts as a point (gene) mutagen in bacterial systems.

1. Microorganism Studies

Investigations by Embree (25) with test strains of Salmonella typhimurium were cited. Three strains were tested. Strain TA1535 was reported to show histidine revertants indicating that mutation by base-pair substitution had occurred. Tests with strains TA1537 and TA1538 were negative, which EPA interpreted as showing that ETO does not induce frame-shift mutations. Studies by Rannug et al. (60) with strain TA1535 were cited to confirm ETO's ability to induce mutation by base-pair substitution. In an addendum to Rannug's paper, Hussain et al.(36) reported the genetic risk (potency) of ETO with Escherchia coli. The authors estimated this risk to be two mutants per 10^8 survivors per mM x hour.

A Stanford Research Institute Study, Kauhanèn (39) established that a dose-response relationship occurs for mutations in S. typhimurium strains TA1535 and TA100. There were negative results in strains TA1537, TA1538, and TA98. EPA stated that

this confirmed that ETO induces mutations by base-pair substitution. A rat liver microsomal activation system was used and did not affect the mutagenic activity. This was reported to indicate that ETO is a direct-acting point (gene) mutagen in microbial systems. It was stated that microsomal activation provides information on the effect which mammalian metabolism can have on the genetic activity of a compound. The possible effects are the conversion of a promutagen to a mutagen and the conversion of a direct-acting mutagen to a nonmutagen. Investigations by Kolmark, et al. (43) (44) and Kilbey (40) with strains of Neurospora crassa were cited as indicating ETO caused reverse mutations in plate and culture media tests.

2. Plant Studies

Seven publications dealing with the effect of ETO on barley, wheat, and rive were cited. It was claimed that the studies provided evidence that such treatment results in heritable, viable mutants among the segregating generations. No details were supplied.

3. Invertebrate Studies

Investigations by Nakas, et al. (53) and Watson (90) were cited. Male Drosophila melanogaster (fruit fly) were injected with solutions of ETO and then mated with females and the progeny examined for

mutations. EPA reports that the increased incidence of these mutations showed a dose-response relationship.

4. Mammalian Studies

Effects on mammalian species included cytogentic studies by Embree (25), Strekolova (76), Strekalova, et al. (77), and Fomenko, et al. (26), with rats; dominant-lethal assays by Embree (25), and Strekolova, et al. (75), with rats were cited as well as one human mutagenic episode retrospective study by Ehrenberg (10).

Mutagenic Effects of ECH

The EPA position document contained references from ten publications concerning the biological effects of ethylene chlorohydrin which would seem to establish the point (gene) mutation potential of ECH.

Mutagenic Effects of EG

The EPA position document cited one study with ethylene glycol that failed to show point (gene) mutation effects.

Reproductive Effects of ETO

EPA regulations (40 CFR 162.11(a)(3)(ii)(B) provide that a rebuttable presumption shall arise if a pesticide "produces any other chronic or delayed toxic effect in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety". Studies conducted with guinea

pigs and rats were cited and judged by EPA to indicate that ETO can adversely affect the male reproductive organs.

BIOLOGICAL AND ECONOMIC INFORMATION BY COMMODITY

A. Apiculture

In the United States, over 90 crops, valued in excess of $8 billion annually require or are benefited by bee pollination. Inadequate pollination can result not only in reduced yields but also in delayed yields and a high percentage of culls or inferior fruits (50). In addition to pollination, honey bees annually produce approximately 200 million pounds of honey valued at $100 million, wholesale. A frequently overlooked product of honey bees is beeswax. About 3 million pounds of beeswax valued at $6 million also are produced annually by honey bees.

Over the last 30 years the trend in the number of colonies of bees has been downward (1). This is the result of urbanization, pesticide usage, clean culture, monoculture, disease, and low honey prices. Inflation has also taken its toll, as the cost of bees and bee equipment has increased over 200% in the last 10 years (2, 3).

Fumigation with ethylene oxide (ETO) is an invaluable tool in the prevention and control of bee diseases (51, 16, 47, 58). Its primary use in beekeeping is as an alternative to burning bee equipment which may be contaminated with disease (68, 70, 63, 42). Ethylene oxide is also effective against European foul brood, chalkbrood, and nosema disease (51, 71, 72, 16). Tests indicate that colonies placed in ETO fumigated hives develop larger populations due to controlling unidentified diseases of the honey bee (71, 47). In addition, pests

such as the greater wax moth, Galleria mellonella, also are controlled by ETO (51, 45).

American foulbrood is the most widespread and the most destructive brood disease of honey bees. It is caused by a spore-forming bacterium, Bacillus larvae. Infection of the bee larvae occurs after ingestion of spores in contaminated food. Infected larvae degenerate into a spore-laden mass that dries to a scale which contains approximately 2.5 billion spores and adheres rigidly to the cell of the honeycomb. These scales become the primary source of reinfection of other larvae since house bees clean the cells and distribute the spores throughout the hive. American foulbrood is spread from hive to hive when B. larvae spores are brought into healthy colonies by bees "robbing" honey from diseased hives or by bees drifting from a diseased colony. This spread is a relatively slow process due to the natural defense mechanisms of a colony. However AFB is spread rapidly when bee equipment which is contaminated with large numbers of B. larvae spores or containing AFB scales are transferred to healthy hives. Colonies that contract AFB die out if the disease is not controlled.

In some areas, European foulbrood (EFB), another brood disease, poses a more serious threat to beekeepers than AFB because it occurs most frequently at the time when colonies are normally building their peak populations. The causative organism, Streptococcus pluton, gains entry into the larvae in contaminated food, multiplies rapidly within the larval gut and causes death about 4 days after egg hatch. The bacteria are

spread in the same manner as with AFB disease. Colonies can be seriously weakened or die if EFB is not controlled.

Chalkbrood is caused by a fungus, Ascosphaera apis, which affects only the brood. After the spores are ingested they germinate and proliferate. The fungus grows out of the larva, covering it with white mycelium. Although the disease normally does not destroy a colony, it can prevent normal population build-up when the disease is serious. Chalkbrood usually disappears or is reduced as the air temperature increases in the summer. The spores remain viable for years and, as with AFB-and EFB-contaminated bee equipment, are the chief source of reinfection.

Nosema disease is a major disease of adult honey bees and can cause extensive losses. The causative organism of the disease is a protozoan, Nosema apis. The protozoan is transmitted by ingestion of its spores, which germinate soon after reaching the ventriculus. The spread of nosema disease occurs chiefly through the use of contaminated equipment, the robbing of infected hives, through infected package bees, or infected queens and their attendant workers.

Method of ETO Application

At the present time, five states, New Hampshire, New Jersey, Tennessee, Virginia and West Virginia, have a section 24(c) registration under the Federal Insecticide, Fungicide and Rodenticide Act for using ETO. Ethylene oxide fumigations in these states are restricted for use under the control of the regulatory agency responsible for apiary inspection.

Also, seven states and the Bioenvironmental Bee Laboratory are using ETO on an experimental basis.

Permanent or mobile chambers are used for performing fumigations. Fumigations are usually conducted in mobile chambers in isolated outdoor areas. The permanent units are located in well-ventilated, unoccupied buildings with the ETO exhausted to the outside atmosphere.

The fumigation procedure generally consists of loading the chamber with bee equipment, sealing the door and evacuating to 26 inches of mercury. A measured quantity of ETO is introduced and the chamber operated for a specified period at approximately 100°F. The chamber is then re-evacuated to 26 inches of mercury and vented to atmospheric pressure. The door is then opened and the equipment removed and aerated for a minimum of 24 hours either in a well-ventilated, unoccupied room or outside.

Dosage and Use

Ethylene oxide usage for control of bee diseases is summarized in Table 1. It is estimated that approximately 1,500 pounds of actual ETO is currently used annually.

Safety and Containment Practice

In normal usage of ETO in gas-tight chambers, the chances of the operator being exposed to the fumigant is minimal. Exposure to ETO can occur while loading or unloading the fumigation chamber. Another exposure could occur at the time the equipment is being moved by the beekeeper. This exposure would be minimized since handling would occur at

least 24 hours after the equipment had been aerated at temperatures in excess of 70°F.

Possible ways to improve safety would be to increase the number of vacuum air washes, use respiratory and protective equipment as recommended by the manufacturer, and inspection or testing procedures of fumigation chambers as prescribed in Section IV Part 1 of the Plant Protection and Quarantine Treatment Manual (85).

Importance of ETO to Apiculture

The losses suffered by beekeepers as a result of diseases and pests of honey bees are difficult to assess. Losses are reflected in a decreased honey crop, fewer bees for pollination, and a drop in the production of beeswax. In addition, various states expend thousands of dollars annually to enforce laws and regulations which are designed to control bee diseases, primarily AFB. These laws attempt to regulate movement and entry of bees, issuances of permits and certificates, apiary location, control and quarantine, inspection and methods of treating diseased colonies. The destruction of AFB-diseased colonies is included in most State laws. In 1963, various states spent an estimated $1 million in apiary inspection (69). In the same year, the value of colonies destroyed under State laws to control AFB exceeded $470,000 (69). Ethylene oxide could have been used to save most of this equipment had it been approved.

The value of a bee hive consisting of a top and botton board, 2 deep and 2 shallow supers with drawn honeycomb is

estimated at $100. The cost of fumigating this equipment ranges from $1.00 to $3.50 depending on the ETO formulations and concentrations used.

Because of the broad spectrum of microorganisms and pests that ETO can control, it can reduce the use of two antibiotics, Terramycin (AFB and EFB) and fumagillin (Nosema disease), and use of ethylene dibromide and paradichlorobenzene which are presently used to control wax moths.

In states where ETO fumigation is available, apiary inspectors have found that beekeepers are more cooperative toward the disease abatement program since their equipment is no longer required to be destroyed. In these states, diseased hives and equipment which previously would have been undetected by the apiary inspectors are being brought to their attention.

Relative Effectiveness of Alternative Controls

The control of AFB is a local option and as such is subject to the laws and regulations of the various states. Some states permit the use of antibiotics in the treatment of this disease. Others forbid their use for the control of AFB and insist upon the destruction of the colonies by burning or decontamination of the hive equipment. In some states, EFB is treated similarly when found.

Disease	Other Controls	Application and Limitations
American Foulbrood	Terramycin -	Fed to honey bee colonies either in 1:1 sugar syrup (200 mg A.I./1.9 l syrup) or as a dust (200 mg in 28 g of powdered sugar) applied directly on the top bars of the brood combs. The use of Terramycin to eradicate AFB is generally not practical as the causal organism is not destroyed. Disease development is controlled only while the antibiotic is present in the larval food.
	Burning -	The destruction of diseased colonies and contaminated equipment effectively eliminates potential sources of infection. However, open burning is prohibited or restricted by local or state laws

Disease	Other Controls	Application and Limitations
		and closed burning facilities are generally unavilable.
	Boiling Lye Solution (sodium hydroxide) -	Hive parts are immersed for 20 minutes in a boiling lye solution (1 lb. of lye to 10 gallons of water). However, only the wooden hive parts can be salvaged and these are usually weakened or damaged by the process.
European Foulbrood	Terramycin -	Same as for AFB.
Chalkbrood	None	
Nosema	Fumagillin -	Fed to honey bee colonies in 2:1 sugar syrup (75-100 mg A.I./1.9 l syrup. Disease development is controlled only while the antibiotic is present in the food.
	Acetic Acid Fumigation -	Hive equipment exposed for 1 week (¼ pint 80% acetic acid per hive body).

There is a lack of agreement as to the benefit of antibiotics for the treatment or prevention of bee diseases. Concern about the use of antibiotics includes the possibility of selecting a strain of drug-resistant bacteria making control more difficult, and the possibility of comtamination of honey with antibiotic residues.

Pest	Other Controls	Application and Limitations
Wax Moth (Galleria mellonella)	Ethylene dibromide Fumigation-	Used only on stored combs containing honey consumption; it is now in the RPAR proceedings.
	Paradichlorobenzene-	Used only on stored combs not containing honey for human consumption, it is being considered for RPAR.
	Low Temperatures-	Exposure to 5°F for 2 hours kills all stages of the wax moth.

Summary

The honey bee industry needs ETO fumigation for treating hive equipment from colonies with American foulbrood (AFB) disease. Over $8 billion of agricultural crops require honey bee pollination. In addition, the honey bee produces honey and beeswax valued at $106 million annually.

Contaminated beekeeping equipment is the principle reservoir for bee disease agents. Ethylene oxide fumigation is used to recycle contaminated equipment or equipment of

unknown disease background. There is no registered alternative material available for this fumigation. Until ETO fumigation was developed, contaminated bee equipment was destroyed by burning. This procedure resulted in the loss of bees and hive equipment. It also helped develop a lack of cooperation of the beekeeper with the apiary inspector. Aside from these considerations, many jurisdictions also restrict open burning and approved closed burning facilities are usually unavailable.

Ethylene oxide fumigation for recycling hives infected with AFB has other benefits. In addition to destroying Bacillus larvae, ETO also kills the causal organisms of European foulbrood, chalkbrood and nosema disease. Also, tests indicate that colonies placed in ETO fumigated hives develop larger populations due to controlling unknown diseases of the honey bees. In addition, pests such as the greater wax moth, Galleria mellonella, are controlled by ETO.

Table 1. Summary of Current ETO Usage for Bee Disease Control in the U.S.

State	Formulation	Annual usage (lbs)[1]	Actual usage/yr (lbs)[1]	ETO/treatment (lbs)[2]	mg/l of chamber space[2]	No. of treatments/yr[1]	Min. exposure time (hrs)	No. personnel involved	Type fumigation chamber	No. of vacuum evacs.
Alabama	10% ETO 90% CO_2	600	60	2	450	40	8	4	Steel Mobile	2
Bio. Bee Lab	12% ETO 88% Freon	650	78	3.6	700	20	16	2	Steel Permanent	1
Connecticut	100% ETO[3]	135	135	3	550	50	24	1	Wood & Fiberglass Permanent	0
Delaware	10% ETO 90% CO_2	180	18	0.35	450	50	8	1	Steel Permanent	3-4
Maryland	10% ETO 90% CO_2	600	60	2	450	30	16	1	Steel Mobile	1
New Hampshire	10% ETO 90% CO_2	500	50	2.5	450	20	8	1	Steel Mobile	1
New Jersey	10% ETO 90% CO_2	3,500	350	3.5	450	100	8	3	Steel Permanent	2
New York	12% ETO 88% Freon	1,000	120	1.8	450	65	48	4	Wood & Metal Mobile	1
Oregon	10% ETO 90% CO_2	600	60	2	450	30	6	1	Steel Mobile	0

Table 1. (Continued)

State	Formulation	Annual usage (lbs)[1]	Actual ETO usage/yr (lbs)[1]	ETO/treatment (lbs)[2]	mg/l of chamber space[2]	No. of treatments/yr[1]	Min. exposure time (hrs)	No. personnel involved	Type fumigation chamber	No. of vacuum evacs.
Tennessee	10% ETO 90% CO_2	1,000	100	2.5	450	40	8	2	Steel Mobile	1
Virginia	50% ETO 50% CO_2	660	330	2.2	1,200	150	4	5	Steel Mobile	2
Washington	12% ETO 88% Freon	200	24	0.36	131	70	24	1	Steel Mobile	1
West Virginia	10% ETO 90% CO_2	1,000	100	2	450	35	8	1	Steel Mobile	1
	TOTAL		1,485					27		

1/ Approximation

2/ Minimum

3/ ETO mixed with CO_2 in chamber to equal 20% ETO - 80% CO_2

B. High Containment Research Laboratories in Agriculture

The USDA maintains several high-containment laboratories devoted to research and diagnosis of domestic and exotic (foreign) plant and animal disease pathogens. As will be developed below, ethylene oxide (ETO) is used in decontamination/sterilization procedures only when no known substitute exists.

In working with these pathogens, two important biological safety aspects are of utmost concern: protection of laboratory workers and protection of susceptible plant and animal populations. For purposes of this report, the human health and safety protection against these pathogens will not be discussed. Suffice it to say that inherent in the operation of these facilities every effort is made to protect all employees. In designing facilities for work with such pathogens, one principal concept is followed: maximize the containment of the pathogen.

At such facilities, decontamination is a general term used to mean complete destruction of (exotic) pathogens. Any one of a number of effective decontamination procedures may be employed, depending on the particular item under consideration and the eventual destination or future use of such an item. Exposure to steam heat, various chemicals, acids or alkalis, or gas vapors are all utilized for specific purposes. Decontamination may not necessarily imply sterilization, but it generally does.

Sterilization is a general term used to refer to a process whereby complete destruction of all microbial organisms has been accomplished. In general, sterilized products are those which are used in animal studies or tissue culture work to ensure nonintroduction of contaminating agents. Exposure to steam heat, various chemical sterilants, gas vapors, or ionizing radiation are commonly used in sterilizing products. Sterilization is an effective means of decontamination.

In considering the decontaminating or sterilizing procedure to be employed, a number of factors are considered, such as type of pathogen, eventual use of an exposed product and intended destination. It is further recognized that the sensitivity of many pathogens, including animal viruses, to decontamination may alter drastically when such pathogens have dried onto surfaces. That is, a virus which might be destroyed rather easily while suspended in a fluid state may become more resistant to the same decontamination protocol when dried in the presence of salts or proteins (73, 81). Because of the highly infectious nature of these pathogens and the policies of the U. S. government to prevent the introduction of such pathogens into our environment, complete decontamination is essential when materials must be removed from these laboratories. The impact resulting from an outbreak of such diseases in our plant or livestock populations would be disastrous to the economy (49).

Ongoing research into effective applicable decontamination procedures has resulted in the formulation of policies

governing movement of materials within the laboratories as well as from these laboratories. Under certain circumstances (outlined below) the only feasible and reliable method of choice is exposure to ethylene oxide (ETO).

ETO has been known as an effective antibacterial agent, particularly against spore-formers such as B. subtilis (75). Additionally, ETO is known as an agent capable of destroying many viruses. Noteworthy examples include eastern equine encephalomyelitis virus (12); influenza A and influenza B viruses, Newcastle disease virus, Columbia MM virus, Theiler's PA mouse encephalomyelitis (30); vaccinia and Columbia SK viruses (41); vaccinia virus (91); herpes simplex virus, parainfluenza virus, and polio virus (73). Even more importantly, ETO effectively destroys the exotic pathogens studied at these laboratories (e.g., foot-and-mouth disease virus (15, 66, 81, 80, 89); various plant pathogens (37). Additional examples are plentiful (e.g., see (24); see also below).

Many of the operational details reviewed in this report pertain to those employed at the USDA Plum Island Animal Disease Center, Greenport, New York, (see further on), but the general concepts are applicable at the other laboratories as well. Ethylene oxide is used for decontamination and sterilization at the following three laboratories:

(1) Plum Island Animal Disease Center (PIADC), Greenport, NY (see Appendix I).

The PIADC is located on a coastal island approximately

1.5 miles northeast from Orient Point on the north shore of Long Island. An island location for this facility was mandated by Congress to minimize the possible spread of exotic (foreign) animal disease agents to the livestock populations of the United States. The PIADC is charged with the responsibility of maintaining diagnostic capabilities for a variety of exotic animal pathogens and for conducting basic and applied research relative to these agents (see Appendix II).

All work with these pathogens is done in one of two high-containment laboratories. Some aspects of these laboratories include: maintenance of increasing degrees of negative air pressure from areas of lesser to greater contamination; high-volume single pass air flow, with all air filtered before discharge; self-contained waste water sterilization facilities; incinerators for disposal of burnable wastes, including animal carcasses. Additionally, all personnel remove street clothes upon entering and wear special clothing in the laboratories; before exiting, decontaminating showers are required. These and other biological control measures have earned the laboratories P3 Biological Containment Status. Upon completion of a change-over from deep-bed to HEPA filtration systems for exhausting air, both laboratories will regain P4 status, the highest rating possible.

(2) National Animal Disease Center (NADC), Ames, IA.

The NADC is located in Ames, IA. Among other

activities, this laboratory conducts basic and applied research on animal disease agents which are currently present in the United States. Diagnostic capabilities for these agents are also maintained.

(3) Plant Disease Research Laboratory (PDRL), Frederick, MD.

The PDRL has the mission to 1) investigate nonresident (exotic) plant pathogens that are judged to represent serious threats to major food crops grown in the United States; 2) introduce and screen candidate bio-control agents; and 3) receive, culture, verify purity, and hold plant pathogens imported under APHIS-approved labels before release to qualified investigators. The major plant pathogens currently under investigation at the PDRL are listed in Appendix III.

Method of ETO APPLICATION

In the high-containment research laboratories, all ETO sterilizers are operated as closed systems: they are vented by direct connection to the discharge air system; the air is filtered by passage through the deep-bed or HEPA systems and discharged through stacks located on the roof of the buildings. Multiple (2 or 3) air purges (self aeration) are used to evacuate ETO after a sterilization run.

Dosage and Use

Laboratory	ETO Formulation	Estimated Annual Usage (pounds)	Estimated Actual ETO Annual Usage (pounds)
PIADC	12% ETO, 88% freon	2500	300
NADC	12% ETO, 88% freon 12	1350	162
PDRL	12% ETO, 88% dichloro-difluoromethane	340	41

At each of these laboratories ETO is purchased in large (e.g. 140#) cylinders.

PIADC Facility

At present, ETO gas sterilizers are located only in the main research laboratory. One large double-ended ETO gas sterilizer is used for removal of certain materials from the interior (contaminated) to the exterior of this laboratory. A Safety Technician operates this unit approximately twice a week. A smaller double-ended ETO gas sterilizer, located in one of the rooms assigned to Safety Research, is used primarily for sterilization of materials for internal laboratory use and, on occasion, for transfer of certain materials from an area of higher contamination to an area of lesser contamination. A laboratory technician assigned to Safety operates this unit approximately once a week.

Additional use of ETO as a sterilant and decontaminant will ensue upon completion of the installation of a new lyophilizing unit in the Cytological Research laboratory. Anticipated additional ETO usage will include a large gas sterilizer in the new Vaccine Production Unit currently under

construction, contiguous with the research laboratory. It is also anticipated that ETO gas may be used in conjunction with a lyophilizing unit in the Diagnostic Laboratory.

NADC Facility

In the total operation of the NADC, there are 19 large combination steam and ETO gas sterilizers, 13 small ETO gas sterilizers, and 3 portable ETO units. In practice, only four of these sterilizers are presently in use. These are 8.2, 93, 148 and 994 liter units. One or another chamber is used, by one of two Safety Technicians, for an average of 4.5 times a week.

PDRL Facility

Building 374, the disease-containment facility, consists of five sealed glasshouses, adjacent laboratories, air handling equipment and temperature control facilities. For removal of materials from these laboratories (contaminated) five double-ended steam autoclaves adapted for use with ETO gas are used. Two or three technicians operate one or more of these units for an average of twice a week.

Safety and Containment

The ETO sterilization chambers located in these high containment laboratories are operated as closed systems (see METHODS OF APPLICATION). The rooms in which these units are permanently installed have single-passage air-flow systems, the air being completely changed 12-15 times per hour.

All ETO gas sterilization operations are performed under the supervision of the various Safety Officers by

appropriately trained Safety personnel.

Importance of ETO Usage in High Containment Laboratories

PIADC Facility - The estimated total formulation usage is 2500 lbs per year. The major, and most important use of ETO at the PIADC is for treatment of materials prior to removal from the laboratories.

The policy of the PIADC is to take appropriate measures to ensure that there is no possibility of introducing exotic animal disease pathogens into the environment of the U. S. It is important to recognize that many of these pathogens (cf. Appendix II) will infect a wide-range of different animals. By definition, exotic pathogens do not exist in the U. S. U. S. policy prohibits vaccination against these agents. Introduction of these pathogens would therefore result in a very rapid, unchecked spread.

For purposes of this discussion, consider foot-and-mouth disease (FMD) virus; essentially similar situations exist for these other pathogens. The last outbreak of FMD in the U.S. occurred nearly 50 years ago. The policy then, as now, was to eradicate FMD by slaughter, burning, and burial. FMD is a disease of cloven-footed animals. Our domestic susceptible livestock (cattle, sheep, goats) numbers approximately 2×10^8 animals. Since deer, for example, are also susceptible hosts (52), the spread of this disease would be explosive in our wild-life populations as well.

Although the U.S. spends approximately $10 million per year for control measures to prevent the introduction of FMD

into the U.S., it is estimated that a major outbreak of FMD could cost $12 billion over a 15 year period (49).

Suffice it to say that comparable monies and control measures are extended by Canada and our Central American neighbors, all of whom are free of FMD. Free of this disease, the U.S., is in a position to engage in unrestricted trade with many foreign nations. It is imperative, therefore, to take every possible measure to contain these pathogens.

From time to time it is necessary to remove certain items from these high-containment laboratories, either for transfer to other locations at Plum Island, or for transfer off the island. Since some of these items are being removed for repair, replacement, or modification, it is important that such items not be damaged. Experience has shown that it is occasionally impossible to have in-house repairs done. It must be emphasized again that, whenever possible, alternative means of decontamination are used. However, for items that are sensitive to heat, steam, the corrosive action of acids or bases, or immersion in other liquid disinfectants, gas vapor decontamination is the only method available. ETO gas is the only known suitable decontaminant/sterilant. Listed in Appendix IV are examples of some of the items removed from these laboratories during 1977. The alternative to removal for off-island repair or modification is replacement.

Because of the necessity for biological containment, USDA has also taken the position that anything leaving the island, even if not from within the laboratories, must be

decontaminated. Examples of such items removed from Plum Island during 1977 after ETO sterilization are given in Appendix V.

Minor uses of ETO at PIADC include: 1) Various heat- or moisture-sensitive devices are ETO sterilized within the laboratory before use. Since such devices are packaged within the laboratory (contaminated area) it is necessary to destroy not only common microbial contaminants but also specific disease pathogens. This is critical for work both within the animal containment facilities and also in tissue culture work. In the preparation of diagnostic (biological) reagents (e.g., specific antigens or antibodies) there can be no interference introducted through cross-contamination by unrelated microorganisms. 2) Similarly, various materials or equipment must be decontaminated after use, cleaned, and then sterilized prior to reuse within the laboratory. The high cost of replacement mandates that certain devices be recycled for additional use after exposure to these pathogens. For example, some of the plastic ware and rubber products utilized in the operation of USDA gnotobiotic animal research unit are recycled between studies by ETO. The lyophilizing units used in preparing diagnostic reagents are decontaminated between runs with ETO to prevent cross-contamination. Similarly, the Multiple Automated Sample Harvester (MASH) is decontaminated with ETO between uses. Optical devices such as cameras or electronic equipment such as telemetric sensing devices are also treated with ETO for decontamination purposes.

3) Film has also proved to be a difficult item to transfer out of a high-containment laboratory (7). Specifically, ETO has proved to be the only acceptable decontaminant for unprocessed film. The PIADC maintains the capability of in-laboratory processing of most black-and-white and color film, excluding movie film. When film has been processed, decontamination by formaldehyde or acid is the standard procedure, since these chemicals do not alter the film's characteristics at this point. Such is not the case for unprocessed film. The PIADC does not have movie film processing capabilities. The alternatives are off-island processing or installation of film processing equipment.

In recent years the PIADC has undertaken the production of about 10 training films on the recognition and diagnosis of exotic animal diseases. These films eventually will be used by APHIS and the veterinary medical colleges. Since the PIADC is the only place in the U.S., where such footage can be shot, the decontamination of unprocessed movie film has become quite important. 4) In addition, a wide variety of sterile "disposable" products are routinely used at the PIADC. Such items include blood-collecting equipment, pipettes, syringes, tips for mechanical dispensing equipment, petri dishes for bacteriological use, and various diagnostic aids such as sampling swabs or tubes. All of these items are supplied by the commercial manufacturers as ETO-sterilized products.

In some instances, such "disposable" items may be recycled (by ETO sterilization) for internal laboratory use as a

cost-saving measure. The operation of efficient, modern diagnostic and research laboratories relies quite heavily on the availability of such commercially-produced materials. Certain procedures require their use, as no suitable substitute exists. Where substitutes do exist (e.g., certain glassware items), cost analysis has indicated that utilizing the "disposable" sterile and pyrogen-free products is the choice.

NADC Facility - The estimated total formulation usage is 1350 lbs per year. ETO usage is rather evenly divided between removal of equipment from the high-containment laboratory and for in-house sterilization of certain items. Examples of items removed from the laboratory include electrical and electronic equipment, pregnancy testers, vacuum pumps, aerosol samplers, typewriters, spectrophotometers, microscopes, clocks, and analytical balances. The alternatives to removing these items is replacement.

For reuse within the laboratory, ETO gas is used to sterilize such items as: pipettes and other plastic ware, surgical gloves, surgical packs containing plastic or rubber items, respirators, various filters, certain clothing items, and laboratory items made of wood.

ETO is also used in the preparation of equipment and items used in gnotobiotic animal studies. In addition to some of the above-mentioned items, halters and other veterinary supplies, animal bedding and feed are also sterilized with ETO.

PDRL Facility - The estimated total formulation usage is 340 lbs per year. The philosophy governing the operation of the PDRL (a P4 containment facility) is similar to that of the PIADC--to prevent introduction of the disease pathogens into the environment from the laboratory.

During the years 1942-1969 an intensive research program was accomplished in the Army Biological Laboratories at Fort Detrick. Through the period there was a continuous effort to improve on gaseous sterilization techniques; however, for general use, ETO was judged to be superior to other products tested. After 20 years Phillips, then Chief of Physical Safety stated, "Ethylene oxide is apparently effective against all types of microorganisms" (56). Since 1971, PDRL has used and is dependent on ETO for sterilization problems. Tests have shown that the plant disease propagules of those fungus pathogens under investigation are very sensitive to low concentrations of ETO and are killed within an exposure period of approximately one hour (37).

In dealing with most of the plant pathogens under study, it is recognized that the host is generally highly specific. Most of these pathogens, when exposed to sufficient moisture, will germinate. If no host is available, the pathogen cannot survive.

ETO gas sterilization has routinely been used at the PDRL for decontamination of materials before removal from the laboratories. When steam sterilization would damage or destroy items such as electronic test equipment, walkie-talkie

radios, camera gear and exposed film, analytical balances, microscopes, etc., ETO gas has successfully been used with confidence.

The capability of plant seeds to withstand ETO exposures adequate to destroy surface born disease propagules provides a valuable tool for the decontamination of seeds grown in the presence of dangerous plant pathogens.

After the soybean rust disease was discovered on Puerto Rico in 1976, a study on the use of ETO to surface sterilize soybean seeds was accomplised in PDRL (37). The proximity of this disease to the mainland causes serious concern. Additionally, large numbers of U. S. soybean breeding materials are cultured on the island during the winter. Seeds produced are then shipped to the States for spring planting. Seed stocks and containers must be decontaminated before shipment from the island. The use of ETO as a technique to eliminate viable rust spores from the materials was investigated and demonstrated in the PDRL containment facility. A one-hour exposure to ETO had little to no effect on the germinability of soybean seeds and successfully killed the Phakopsora pachyrhizi spores.

In similar studies in Australia, the effectiveness of ETO to sterilize diseased and contaminated seeds and leaves was investigated (59). In tests leaf-trash samples were completely sterilized, the concentrations of viable bacteria from infections on beans were sharply reduced and fungal pathogens on the surfaces of seeds were inactivated. It was concluded that

ETO shows much promise for use in establishing foundation seed plantings free of seed-borne bacterial and fungal pathogens.

The findings in these studies indicate that ETO decontamination techniques can be developed for use on seed stocks to permit their introduction into the U.S. sans exotic plant disease propagules.

Importance of Maintaining ETO in Agriculture Laboratories

As a decontaminant ETO has proven to be an extremely useful and reliable product. Used according to the specifications for safe handling recommended by the manufacturers of the gas sterilizers, ETO destroys a wide variety of plant and animal pathogens. As indicated above, various electrical, electronic and optical devices can be removed from high-containment laboratories knowing that these devices will not serve as fomites for pathogen escape.

As a sterilant, ETO is likewise very important in the preparation of a number of instruments, plastic components and mechanical devices which are subsequently used within the various laboratories. In diagnostic procedures, it is necessary to prepare appropriate antigens, antibodies or other biological reagents which are highly specific. Cross-contamination by extraneous microbial agents could result in expensive erroneous conclusions. If preparing a series of lyophylized viral antigens, for example, the machinery must be sterilized between runs. Considering that with foot-and-mouth disease virus there are seven major antigenic types and at least 64 recognized subtypes, it is clear that the specificity of

preparing "clean" antigens is very important.

Should lyophilized antigens subsequently be utilized in the preparation of vaccines, such specificity is equally important.

Thus, ETO continues to play an irreplaceable role in various phases of critical research and diagnosis. At this point there is no acceptable substitute for all conditions outlined herein (see below). Ongoing research into a wide variety of agents suitable for use as decontaminants or sterilants effective against these pathogens may result in acceptable alternatives, but none tested so far have met all the criteria of efficacy, reliability and safety.

Impact on Productivity and Operation of High-Containment Laboratories

In general, loss of ETO as a sterilant at the PIADC, NADC, and PDRL would seriously compromise the biological safety standards. Having to use alternative sterilants would result in the loss by destruction of a wide variety of tools and instruments which are either recycled for within-laboratory use (see above) or which need to be removed from these laboratories (Appendix IV, V). Determining replacement costs for all such items is difficult, but examples are presented in the appendices, A minimum estimate of the value of items removed from the PIADC laboratories for off-island repair or use at a different island location during 1977 was about $23,600. Additionally, the value of items removed from the island for repair or modification which did not originate in the laboratories was at least $10,800.

In some instances, it might become necessary to replace certain heat-sensitive plastics and rubber items with glass or metal substitutes. Cost estimates are impossible to ascertain since, in some cases, such substitutes do not exist and would have to be custom made.

Many of the commercial "disposable" ETO sterilized plastic materials which are used within the laboratories are ordered directly by the individual units and cost estimates are difficult to obtain. One diagnostic unit ordered $2,665 worth of such products during the 6-month period October 1977-March 1978, or slightly more than $5,000 per year. If all five research/diagnostic units had similar use, then approximately $25,000 per year becomes a minimum estimate.

At the PIADC during the past year the cost of disposable pipettes was $800.

These figures do not begin to reflect those items which are cleaned and sterilized (by ETO) for in-house reuse.

Mention is made again of the fact that certain film which is exposed within the animal containment facilities at the PIADC must be removed undamaged from the laboratories. Since there are no other facilities available in the U.S. where such documentation is possible, it is impossible to attempt to fix reasonable cost estimates on the value of this footage. These training films are made available to veterinary schools, state and federal veterinarians and others involved with exotic disease recognition or control. As such, these films are an

integral part of training for early diagnosis of exotic animal diseases to prevent their introduction to or spread within the U.S.

ETO decontamination and sterilization of laboratory equipment between use (exposure) with exotic pathogens is frequently used. Examples are: lyophilizers (about $4000, ETO treated twice a week); MASH units (about $1500, ETO treated once a week); and cameras (about $1500, ETO treated twice a month). It would not be economically feasible for such expensive equipment to be purchased on a one-use-and-discard basis. Similarly, cross-contamination is an unacceptable alternative in preparing biological reagents, conducting cell-mediated immunity research or large animal infectivity studies.

The available budget for all laboratory equipment purchases in FY 1978 at the PIADC is $113,700. Replacement of these items would therefore drastically reduce the availability of funds for new equipment.

Assessment of Animal and Plant Disease Introduction

Assessing the impact of the introduction of these various plant and animal pathogens on the U.S. economy is extremely difficult. Where a given disease may be endemic in some other country, often more than one disease may be prevalent, thus making it difficult to determine the impact of only one such disease.

There have been some recent studies pertaining to the economic impact of foot-and-mouth disease in the U.S. (49).

The summary and major conclusions of this study were:

1. If FMD is introduced into the U.S. and leads to a serious epidemic followed by an endemic situation with only voluntary control, the discounted present value losses (mainly in the form of increased consumer costs for animal products) for a 15-year period is estimated to be almost $12 billion.

2. The present policy of restricting the import of animals and animal products is overwhelmingly justified by a benefit-cost ratio of 120 to 1.

3. The strict slaughter and quarantine policy against FMD would be within limits of economic feasibility even to the point where as high as one percent of the livestock was slaughtered in the eradication effort. Such a massive eradication program still yields a benefit-cost ratio of 7.5 to 1. For eradication efforts in which a lower number of animals would have to be slaughtered, say, 0.1 percent (as in the 1914 outbreak), the benefit-cost ratio would be considerably higher and the costs would be almost entirely in the form of direct program costs.

4. From the standpoint of its economic evaluation, the area vaccination approach to eliminating FMD (with a benefit-cost ratio of 16.6 to 1 for our example) would appear to be a feasible alternative following the "stamp-out" policy. But, serious questions might be raised about the technical and political feasibility of containing a large area of the U.S. in a disadvantaged marketing position for the extended time period of three years or more. Also we question the

feasibility of containing the FMD virus in such as area given the contagiousness of the disease and the potential incentives for illicit transport of animals and other carriers of FMD virus to areas with susceptible animals.

5. The relative low benefit-cost ratio estimated for compulsory vaccination implies that there could be a high payoff for new technology leading to improved FMD vaccines and to their more efficient application.

Furthermore, it has long been recognized that there would be serious economic problems resulting from the currently accepted procedures for depopulation of infected and exposed animals. A multi-agency work group was established in 1973 to consider the feasibility of conserving animal protein for human or non-human (pet or livestock) use from animals exposed to certain diseases (6).

Without enumerating all the details involved, existing facilities for transportation, slaughter, processing, rendering and storage were considered inadequate to make conserving animal protein a realistic economic alternative to the current methods of disposal of exposed animals. It was further felt that strong psychological objections to such a product would make public acceptance (either for human or pet consumption) difficult to obtain, thereby defeating the purpose of the animal protein conservation program.

An additional problem associated with assembling economic data for projections of the impact of exotic diseases in the U.S. is the fact that such data is often not collected in

other countries. Some information relative to the pathogens currently studied at the PDRL (Appendix III) are presented here:

(1) Fungi: There are six species of the Sclerospora fungus that attack corn - three are being studied by PDRL. The S. philippinensis on corn in the Philipines frequently destroys 15 to 40% of the crops. S. sorghi attacks sorghum and corn and does occur in the U.S. The disease is epidemic in Venezuela and losses were so great in 1975 that a national emergency was declared (27). S. sacchari was very destructive on corn in Taiwan in the period 1960-74, but damage has been reduced by the use of resistant varieties of corn and sugarcane (79). U. S. corn varieties are highly susceptible to this pathogen (9).

(2) Soy Bean Rust: In the Eastern Hemisphere soybean rust is considered to be a serious disease particularly in tropical and sub-tropical areas. In Taiwan, Thailand and East Australia it is the most economically important fungal disease of soybeans. In southern Japan prior to 1960 losses from rust amounted to 15 to 40% of the crop in individual fields. In 1966 annual losses were 20 to 30% of the crop on Taiwan. In 1968 losses due to the rust were 70 to 80% in some fields on Taiwan. In Thailand in 1971 losses ranged from 10 to 30% for adapted varieties, with complete losses from some introduced varieties. In Australia in 1973 diseased fields near Lismore and Coffs Harbor of New South Wales were complete losses. Losses up to 60% were obtained in tests in the PDRL containment

facility (48). In 1961 the entire U.S. soybean germ plasm collection, approximately 3,000 accessions, was planted in Taiwan and subjected to endemic rust. Only two accessions showed appreciable resistance to rust.

(3) Corn Rusts: There are three important rusts in corn - "common rust", Puccinia sorghi, "southern rust", Puccinia polysora, and "tropical rust", Physopella zeae. Puccinia polysora appeared in West Africa in 1949 and spread rapidly and destructively over the entire country. Damage has been reduced by use of resistant varieties. The disease appeared in the U.S. in 1972. In greenhouse tests in PDRL, yields were reduced 46 to 53%. In field tests losses ranged from 23 to 100%. Puccinia sorghi has caused serious crop losses in sorghum and corn in the southwest. In field tests at Frederick yield losses have ranged from 8 to 10% of the crop

Relative Effectiveness of Alternative Controls

Alternative	Limitations and Applications
Steam Autoclaving	Excellent sterilant; moisture- or heat- sensitive materials destroyed by steam vapors and high temperatures; routinely available and utilized for glassware, disposable materials, liquids, laboratory clothing; exposure time variable (15 min-16 hr) depending on materials; requires permant installation of single- or double-door units.

Alternative	Limitations and Applications
Dry Heat	Excellent sterilant; heat-sensitive materials destroyed; used as an adjunct to steam autoclaves particularly for sterilizing glassware; requires permanent installation; exposure times 4-16 hr; requires heat-up time and uninterrupted operation.
Gamma radiation	Generally excellent surface sterilant; use of gamma-ray source, such as cobalt 60, requires highly sophisticated control procedures, monitoring, and specifically trained personnel; not readily available; not applicable to materials sensitive to radiation; not applicable to many porous materials; gamma rays will not sterilize interiors of most metallic objects.
Ultraviolet light	Limited application as a surface sterilant only, not applicable for use with packaged materials, etc.
Acids/Bases	Depending on the pH sensitivity of the microorganism, may be acceptable decontaminant; both acids and bases are corrosive to many metals and require copious water washing; not

Alternative	Limitations and Applications
	applicable for acid-, base- or liquid-sensitive materials.
Formaldehyde	Used as a liquid decontaminant for some heat-sensitive materials (e.g., processed films); requires total immersion for 15 min to 1 hr; not applicable to moisture-sensitive materials. Used as a vapor for decontaminating interiors of large-volume equipment such as biological containment hoods; generally requires 1 hr exposure in closed container. The residues left by liquid or vaporized formaldehyde must be chemically neutralized and/or flushed with copious volumes of water, followed by air washes. Threshold limit for fumes is 3 PPM.
Paraformaldehyde	Used as a vapor phase decontaminant for large items which can be enclosed in plastic or otherwise sealed; the fumes are flammable and considered eye, skin and respiratory irritants. At the PIADC, paraformaldehyde vapors are used in the sterilization of some gnotobiotic animal equipment, milk

Alternative	Limitations and Applications
	processing equipment and for the complete decontamination of trucks and other vehicles which must leave the island. Extensive aeration and copious water washing required to remove residues.
Peracetic acid	Combustable liquid; can explode at 110°C; powerful oxidizer, may explode when mixed with readily oxidizable materials or chemical accelerants; shock and heat sensitive. Extremely corrosive to all metals, including stainless steel. Some limited use at the PIADC in gnotobiotic animal studies.

Although these various decontaminating procedures (and many others) have been tested against many of these exotic pathogens, and indeed several procedures are used rather extensively in the operation of these laboratories, these alternatives cannot be used exclusively. In decontaminating electronic, electrical or optical equipment as well as exposed unprocessed film, some of the alternative procedures cannot be used. ETO gas has proven to be the only acceptable decontaminant/sterilant (see Appendix IV and V for examples).

One alternative chemical procedure which is used rather extensively at the PIADC is vaporized paraformaldehyde. Long

periods of aeration are required as well as extensive washing of materials affected by the residues. Interior areas of covered surfaces and certain porous materials are not adequately sterilized. The emulsion of film is destroyed by paraformaldehyde (7).

Summary

1. There exists no known substitute to ETO for the specific purposes outlined above.

2. For materials which must be removed undamaged from these high-containment laboratories, but which must not be fomites for exotic pathogens, ETO offers the only currently reliable method of decontamination. High temperature, corrosiveness or high moisture content rule out all other potentially applicable decontaminants/sterilants.

3. Given that there is no substitute, the alternative to removal would be replacement.

4. Furthermore, for certain laboratory equipment needing decontamination between use, it is obviously impractical to consider a single-use-and-destroy alternative.

5. The biological containment mandates governing the operations of the facilities cannot be compromised. Introduction of these pathogens into U.S. environment would clearly be measurable in billions of dollars.

The continued use of ETO as a sterilant and decontaminant in high-containment laboratories is essential. Until other means for destroying these pathogens are developed and proven to be as economical, reliable and as easy to use for these

very specific purposes, ETO appears to be the only choice.

When dealing with the wide spectrum of exotic disease agents, recognizing that their introduction into the U.S. plant, domestic livestock, wildlife, or, in some instances, the human populations would have serious consequences, every means possible to contain such pathogens is vital.

C. Plant Protection and Quarantine Programs

To prevent the entry, establishment, and spread of foreign plant pests into the United States, ethylene oxide (ETO) is utilized as a fumigant/sterilant by the U. S. Department of Agriculture (USDA) as a quarantine treatment. Ethylene oxide is used as a fumigant for snail contaminated cargo and as a sterilant for the control of certain plant disease organisms. The application of treatments required at ports of entry to protect American agriculture is in accordance with legislative authority delegated to the USDA.

Legislative Authority

The Plant Quarantine Act of 1912, as amended (7 U.S.C. 151-167), provides the legal basis for the development of our present day quarantines. The Federal Plant Pest Act, approved May 23, 1957, (7 U.S.C. 150 aa-150 jj) prohibits the importation or movement of plant pests and articles that might harbor the organism. Plant pests as defined in the Act include any living stage of insects, bacteria, fungi, viruses, snails, nematodes, or any other organism that can directly or indirectly cause plant disease or injury to plants, plant parts, or plant products, including those processed or manufactured. The responsibility for the enforcement of these Acts is delegated to the Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine Programs (PPQ).

1. Quarantine Treatment: Fumigant for Snails

Ethylene oxide used as a 10% ethylene oxide and 90% carbon

dioxide mixture (Carboxide^{R}) and methyl bromide are employed as fumigants especially for snail contaminated cargo entering the United States. Following the interception in June 1958 of the very resistant estivating stage of Cochlicella barbara L. (Helicellidae), and several species of other snails on large quantities of United States military cargo returning from Mediterranean areas, fumigation under tarpaulin (gas proof sheets) was used by the USDA to prevent such pests from gaining entry.

Most snail interceptions at ports of entry have been found to occur on retrograde military materials. The location of U. S. Department of Defense installations throughout the world has exposed weapons systems to the native snail populations at overseas installations. Many of these snails are known to be serious agricultural pests which at the present time do not occur in the United States. Infestation and contamination of supplies, support equipment and components of weapons systems are likely to occur and have been found to exist on numerous occasions. Over 23 species of land snails of economic importance have been associated with retrograde military cargoes. Those most commonly intercepted are members of four genera: Achatina, Cochlicella, Helicella, and Theba. The species most frequently encountered is the white garden snail, Theba pisana. These species generally occur in the Mediterranean regions of Europe, Africa, and Asia Minor.

Under the present Defense concept of logistic management of complex weapons systems, components are turned to the United States for modification and repair. If the cargoes arrive at ports of entry infested or contaminated with snails of quarantine significance, the cargoes are placed under quarantine by the Plant Protection and Quarantine Programs, U.S. Department of Agriculture, until decontamination has been accomplished. Fumigation under a gas-tight tarpaulin is the recommended procedure for decontaminating snail-infested cargoes.

Methyl bromide is the preferred and most economical fumigant to use. It, however, has deleterious effects on certain materials causing damage and the development of objectionable odors. Methyl bromide is not corrosive to most metal; however, it attacks aluminum and magnesium and their alloys (Chemical Safety Data Sheet of the Manufacturing Chemists Association for methyl bromide, No. SD-35, 1968). Undesirable off odors may result from a reaction of the fumigant with certain sulphur compounds that have been added to products during the manufacturing process.

Fumigation with the ethylene oxide mixture, Carboxide, was thus developed by the USDA for use on many military and other cargo found contaminated with snails of quarantine significance (62).

Recognizing that methyl bromide has deleterious effects on materials containing rubber, increases the rate of deterioration of certain metal components, and has not been approved for the decontamination of electronic equipment, the military

services require that the ethylene oxide mixture, Carboxide, be used to fumigate material subject to damage by methyl bromide (5).

Methods of Application

The fumigant Carboxide is usually marketed in steel cylinders containing 30 to 60 pounds of the 1:9 mixture. While fumigations may be conducted in approved chambers, most quarantine treatments are conducted under gas impervious tarpaulins because of the lack of commercial treatment chambers at the ports of entry. Either polyethylene film (0.006 inch thickness) or vinyl-coated nylon enclosures are suitable for tarpaulin fumigation. The fumigant is introduced into the fumigation enclosure through metal tubing. The cover is sealed around the perimeter of the enclosure through the proper placement of sand snakes (sleeves or tubes generally made of canvas or heavy gauge polyethylene and filled with sand). Dosages are accurately introduced by weight according to the volume of the enclosure. Fumigant concentrations, distribution, and sorption during fumigation are monitored during the exposure period by USDA personnel with thermal-conductivity gas analyzers. Gas detector tubes are also utilized in the working area. At the end of the fumigation the fumigant is released into the atmosphere. The tarpaulin is not completely removed until the gas analyzer or ETO detector tube readings are negative. These fumigations are generally conducted outdoors in an open area. The area is posted with warning placards. If conducted within a warehouse, only

personnel involved with the fumigation are permitted in the area. Respiratory protective equipment is available and utilized at the fumigation site as required.

The tarpaulin fumigation (1) allows very large amount of intransit cargo to be treated expeditiously, (2) reduces the risk of pest spread by fumigating near the cargo discharge area, and (3) the procedure is easily adaptable to many situations.

Quarantine fumigations are conducted by commercial pest control operators (certified pesticide applicators) under the procedures and supervision of the United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Programs. Treatment schedules and procedures are listed in the Plant Protection and Quarantine Programs Treatment Manual under Section III, Part 1 and 4; Section VI, T402 and T403, (85). Recommendations in this Manual which involve the use of pesticides concern products which have been registered under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, or have been proposed for approval by the Environmental Protection Agency as supplemental labeling for use only in connection with Federal-State quarantine pest control programs.

Dosage and Use

Quarantine fumigation schedules for snails range from 20 lbs of the 1:9 mixture per 1000 ft^3 for 24 hours exposure to 27½ lbs of the mixture for 72 hours (85). Actual ETO dosage is 2-2.75 lbs/1000 ft^3. The added carbon dioxide serves to

reduce the fire and explosion hazard of ETO.

In calendar year 1976, 23 tarpaulin fumigations (ca 174,000 cubic feet) were conducted for snail contamination using 4,356 pounds of the ethylene oxide-carbon dioxide mixture. In calendar year 1977, 64 tarpaulin fumigations (ca 287,000 cubic feet) were conducted using 7,185 pounds of the mixture. Fumigation costs vary depending upon the quantity of material to be fumigated and the size and number of fumigation enclosures required. The commercial fumigator charges average $800.00 per stack enclosure. The USDA charges for the services of PPQ officers whenever fumigations are conducted outside of the regular working hours. The exact number of ETO quarantine fumigations conducted in chambers is unknown. Based on estimates received from commercial operators, fewer than five quarantine treatments per year are conducted in chambers.

Other Quarantine Fumigant Uses

An ethylene oxide fumigation schedule (Plant Protection and Quarantine Program Treatment Manual T310) was developed by Roth (65) for the quarantine control of ticks (Acari:Ixodidae). Ticks of quarantine significance are intercepted on or with various imported commodities of commerce. Methyl bromide is the recommended fumigant for tick control. The development of an ETO schedule, however, provides for an alternative fumigant for methyl bromide. For tick infested materials, 20 lbs of the ETO mixture for 16-24 hours is recommended. Plant Protection and Quarantine records indicate that ETO has not been used in quarantine tick fumigations during the last three

years.

Recent investigations were conducted by Richardson (61) in cooperation with the Australian Plant Quarantine Service to develop information on wood penetration by the ethylene oxide-carbon dioxide mixture gas and its possible use against quarantinable termites, wood borers, and other insects, particularly in overseas freight containers. From the results it appears that the current USDA fumigation schedule for snails should have high wood penetration and insecticidal efficiency against the termites and other insects tested and may be effective against other quarantinable wood borers. Studies on the successful use of ethylene oxide for controlling insect infestations in ancient wooden artifacts were reported by Dominik, et al (22). In the conservation of wood, in special circumstances and particularly in the case of historical objects, the use of a fumigant such as methyl bromide is not always possible as this fumigant causes a change in appearance through discoloration.

Exposure Conditions in ETO Fumigations

The fumigation site is generally outdoors physically separated from work areas. If conducted within an indoor area, ventilation is provided in the area and all personnel not engaged in the application of the fumigant are excluded from the fumigation and adjoining area.

Approved respiratory protective equipment is utilized at the fumigation site as required. The Occupational Safety and Health Act, 29 CFR 1910.134(a)(2) "Respiratory Protection"

presents requirements for the establishment and maintenance of a respiratory protection program. The user of such equipment must be instructed and trained in the proper use of respirators and their limitations [OSHA, 29 CFR 1910.134(b)(3)]. The National Institute for Occupational Safety and Health is the official agency responsible for testing, approval, and certification of respiratory devices.

The American National Standards Institute subcommittee on respiratory protection against fumigants is in the final stages of developing a Standard "Practices for Respiratory Protection Against Fumigants". The Standard which will be published by the American National Standards Institute, 1430 Broadway, New York, NY, will state what type of approved respiratory protection equipment will be used for various fumigants and at what stage of operations during the fumigation. These standards will be adopted by the U. S. Department of Labor/OSHA as acceptable procedures.

Fumigations are now monitored by thermal-conductivity gas analyzers from areas considered remote from a hazard. Recently developed portable infrared analyzers sensitive to the detection of ETO levels as low as 0.4 ppm are now available. Instrumentation can assure safe working conditions at the current 50 ppm TLV level with sufficient sensitivity for good accuracy at much lower levels, should the OSHA limit be lowered.

In 1977, 64 quarantine tarpaulin fumigations were conducted with the ETO-carbon dioxide mixture. It is estimated that

fewer than 300 persons were involved in all of these fumigations. In 1976, 23 fumigations were conducted with an estimated 115 persons involved in the activity.

Relative Effectiveness of Alternative Controls

The USDA has tested all commonly used fumigants against land snails (62). Three fumigants, methyl bromide, hydrocyanic acid (HCN), and ethylene oxide have been found to be the only effective chemicals.

Alternative	Limitations and Application
Methyl Bromide	As previously stated, methyl bromide can cause damage to certain snail contaminated materials.
HCN	HCN, while still effective, cannot be used safely under tarpaulin conditions. HCN is only approved for quarantine treatments in vacuum chambers. Vacuum chambers are not available at most ports of entry. The fumigant is not readily available because there is only one known source (Fumico Inc., Amarillo, Texas) in the United States. In addition, cylinders containing HCN have a shelf life of six months, after which they must be returned to the manufacturer for disposition. The chamber dimensions also restrict the size of load that can be fumigated.

Alternative	Limitations and Application
	Some snail infested equipment includes military vehicles, tanks, armored cars, rocket containers, shell casings, aircraft parts, aluminum landing mats, military engines and other machinery.
Non Chemical	The uses of non-chemical heat and cold treatments have been investigated by the USDA (Unpublished data - USDA, APHIS). The use of heat has not proven practical. High levels of heat unless prolonged, do not provide quarantine control. The use of steam jennys for treating miscellaneous cargo items has not proven effective because the shell protects the snail from the immediate effects of the steam. Low temperatures have shown to be effective for certain species of snails by a 24 hour treatment at 0°F. Refrigerated warehouses and containers, however, with the capabilities of reducing and holding temperatures at or below 0°F. are only available in limited locations. The design of commercially available

Alternative	Limitations and Application
	facilities could not accommodate the size and weight of the cargo. Furthermore, the necessary safeguards to prevent pest escape would be extensive and not practical in most instances. Commercial facilities would not wish to have infested material moved within their premises.

Assessment of Snail Introductions

The damage done by land snails to crops and ornamental plants wherever they have been introduced has run into millions of dollars (55). Some examples include the snail, Helix pomatia, which was reported to damage young vine plants especially in middle and southern Europe. Helix aspersa, a Mediterranean and West European species, injures garden crops such as cabbage, beans, peas, and tomatoes. In California this snail has been observed feeding on leaves and fruit of citrus. Cepaea nemoralis and C. hortensis are sometimes troublesome in gardens in England, also on clover, alfalfa, and pasture land. Helicella candidans, a southeast and middle European snail, is found in clover and alfalfa on the drier slopes. Theba pisana, the white garden snail, is found in vegetable gardens and on young foliage and fruits of citrus in Mediterranean countries and South Africa (86). In the late 1960's, T. pisana was found in the Manhattan Beach area of the County of Los Angeles, California. The State cost for the

white garden snail eradication program for this localized introduction was $30,219 for the period 1966-1971. The application of molluscicide bait was the main method of chemical control. Additional costs of near $10,000 was required for surveys in 1972 and 1973 to verify that the eradication was achieved. California citrus acreage in 1970 was reported near 210,300 acres. The annual citrus treatment cost with chemical baits was estimated to be $10.00 per acre should T. pisana become established in southern California. If all citrus acreage was infested and treatment was required, total treatment costs could be $2,103,000 annually (87).

One of the most serious threats to this country in recent years has come from the giant African snail, Achatina fulica. This voracious eater, with an enormous reproductive capacity, began its immigration from East Africa via human agencies about the turn of the 19th century. In the intervening years this snail has spread to India, Ceylon, the mainland of China, and the East Indies. Its dispersal in the Pacific Islands, nearly denuding some of them, was greatly facilitated during World War II by the rapid conquest of this area by the Japanese. They introduced the snail as a supplemental food source to many new places including New Guinea, New Britain, and New Ireland. The snail was introduced into Hawaii in 1936 and has subsequently cost the taxpayers some $200,000 for control measures, not accounting for the damage to plants in the area. In 1948 it was brought to California on returned war equipment, but an intensive campaign prevented its

establishment (13).

An outbreak of the giant African snail occurred in Florida in 1969. It was declared eradicated in 1975. Eradication treatments included the use of the application of molluscicide baits. Over $600,000 was spent eradicating this introduction.

Molluscicide baits are registered by the EPA for the control of snails and slugs. Based on discussions with domestic producers annual sales for molluscicides within the United States is estimated to be 10 million dollars. Farm Chemical Handbook, 1978, lists 15 basic producers of molluscicides.

2. Quarantine Treatments: ETO Sterilization

Sterilization with ethylene oxide, steam, and dry heat are effective treatments for the control of plant disease organisms. Sterilization treatments are authorized by the Plant Protection and Quarantine Programs to allow industry and other importers the opportunity to import plant materials which would normally be prohibited entry because of the disease risk under the various quarantine regulations. The plant material may include seed, grain, or other plant parts which are not intended for propagation. The purpose of the imports are for trial processing tests with machinery, food testing, or chemical analysis.

ETO is authorized only for non-food imports. Dry or steam heat sterilization is authorized for imports intended for both food or non-food testing purposes. Dry heat or steam,

however, cannot be used on all products because the high temperatures and moist heat can render the material useless for the intended test purpose. For example, dry heat can cause shriveling, brittleness, and other degradation effects. ETO will not affect the size, shape, or physical appearance of the material.

Method of Application

Sterilization treatments are conducted in vacuum chambers. The 12% ETO and 88% dichlorodifluoromethane mixture, usually referred to as the 12-88 mixture is used. ETO is introduced while the chamber is under a vacuum. At the completion of the sterilization period the ETO/air mixture is then safely exhausted. Using the vacuum pump, 3-5 cycles of air introduction and evacuations are repeated. This is a process referred to as "air washing". The doors to the sterilizer are not opened until the completion of the cycles.

Dosage and Use

The present dosage schedule is 25 lbs. ETO per 1000 ft^3 (400 mg/l) for 24 hours at 70°F. or above.

Most sterilization treatments of imported products are accomplished by steam or dry heat treatments. Plant Protection and Quarantine Programs has 15 plant inspection stations equipped to conduct steam or dry heat treatments. The average number of heat treatments is estimated to be less than 500 each year. Only three stations are equipped to use ETO. The average number of ETO treatments is estimated to be less than

25 each year. Imports treated may vary from five to fifty pound shipments.

Exposure Conditions in ETO Sterilizations

In normal usage of ethylene oxide in gas tight chambers or autoclaves the chances of the operator being exposed to the sterilant is slight. Adequate use of a vacuum pump to "air wash" and to remove the sterilant to the outside atmosphere following the exposure period removes the hazard of exposure to the operator. Properly vented room aeration equipment can remove any desorbing gas.

Relative Effectiveness of Alternative Controls

Alternative	Limitations and Applications
Dry Heat:	Dry heat while effective is not an alternative for all imports because this treatment can damage the commodity to such an extent that the material is unfit for its intended usage.
Steam Heat:	Steam heat while effective is not an alternative for all imports because this treatment can damage the commodity to such an extent that the material is unfit for its intended usage.
Formaldehyde:	Paraformaldehyde in the form of a solid polymer of formaldehyde gradually gives off gaseous formaldehyde at ordinary temperatures. When heated it depolymerizes rapidly, giving off

Alternative	Limitations and Applications
	formaldehyde and a little water vapor. The technique results in sterilization of all well-exposed surfaces but it is difficult to sterilize surfaces covered in any manner or to sterilize throughout porous materials. Prolonged airing, often several days, is required to remove the adsorbed surface film of polymer, which continues to release formaldehyde gas slowly.
Radiation:	The only other dry, low-temperature alternatives for sterilization, are radiation sterilization, ultraviolet light, or an electron accelerator. However, ultraviolet light does not penetrate most materials and therefore can only be used to sterilize surfaces of materials. Electron accelerator radiation does penetrate materials well, but only very thin materials. Gamma-ray sterilization, which is usually carried out by exposing the product to radiation from cobalt 60 is the most effective

Alternative	Limitations and Applications
	type of radiation sterilization. There are many disadvantages to this method including the lack of availability of equipment at ports of entry, infrequency of use (see under Use), and it is relatively expensive compared to ethylene oxide.

Summary

Methyl bromide and ethylene oxide are the two fumigants recommended by the USDA for treating snail contaminated cargo. Methyl bromide is the preferred and most economical fumigant. Methyl bromide, however, has deleterious effects on certain materials causing damage and the development of objectionable odors. Snail interceptions at ports of entry have been made on retrograde military materials. Some retrograde cargo that could be damaged by methyl bromide, must be fumigated with ethylene oxide. Fumigations are conducted by commercial pest control operators (certified pesticide applicators), under the supervision of USDA personnel, at fumigation sites where little or no exposure is experienced by the applicators. If ETO is canceled for quarantine fumigation purposes, the only alternative would be to refuse entry to infested shipments into the United States or fumigate with methyl bromide. The damage which could occur from the use of methyl bromide could result in the destruction of valuable equipment and materials. The possible corrosive effect on ammunition, aircraft parts, and

other material would be of great concern to the Defense Department. The safe use of such fumigated items would be questionable.

Dry heat or steam sterilization treatments cannot be used on all intended imports. Sterilization with ethylene oxide provides for the entry of seeds, grains, and other plant parts for non-food use without physical damage to the commodity. Without the continued use of ethylene oxide, requests to import would have to be denied because of a lack of a suitable sterilization treatment.

D. Stored Products

A major use of ethylene oxide (ETO) for stored products includes the spice industry. Spices and natural seasonings are carriers of bacteria, molds, and yeasts in a dormant state. The spice industry fumigates 30 or more different spices and herbs prior to packaging for commercial food processors and for marketing in retail stores. These spices are grown chiefly in tropical regions of the Orient, the main countries of production being the Netherlands East Indies, India, China, Japan, the Malay Peninsula, and certain islands off the coast of Africa, notably Madagascar, Pemba and Zanzibar (33). Spice production is on small farms and the method for handling these spices as they move through the marketing system from production to the export dealer is favorable for development and growth of microorganisms. The volume of spice imported by the U. S. in 1977 was 141,953 million pounds with an import custom value of 132 million dollars (23). To reduce the number of living microorganisms, the 72 member spice industry in the U. S. fumigates annually about 100 million pounds of whole and ground spices and natural seasonings with ETO. The pounds of actual ETO used in these fumigations is about 0.75 million (14). None of the spices or natural seasonings contain any added salt mixture.

Black pepper is the fruit of Piper nigrum (L.) and is one of the more heavily contaminated spices processed by the industry (35). Christensen et al. (18) from the University of Minnesota reported cultures from 30 samples of ground

pepper yielded an average of 39,000 colonies of fungi per gram and the average number of bacteria per gram was 194,000,000. Among the fungi from both black and red pepper were Aspergillus flavus and A. ochraceus and among the bacteria isolated from ground black pepper were Escherichia coli, E. freudii, Serratia sp., Klebsiella sp., Bacillus sp., Staphylococcus sp., and Streptococcus sp. A recent study conducted for the military concluded that with the exception of the bacterium Clostridium perfringes there was no potential health hazard associated with spices and herbs (57). Work reported by Hall et al. (35) states "Ethylene oxide fumigation is an extremely effective means of reducing microbiological populations in all cagetories". Therefore, spices fumigated with ETO are potentially freed from harmful microorganisms whether they be bacterial or fungal. Although no hazard to health is posed by the presence of bacteria and fungi in spices, large microbial numbers added to foods from spices during food processing are considered a violation of good manufacturing practices regulations as set forth by the Federal Food and Drug Administration. By reducing microbial numbers in spices food spoilage is mitigated and shelf life of food is increased, thus reducing energy use and keeping food costs down.

Insects found in spices are the cigarette beetle, Lasioderma serricorne, the flat grain beetle Cryptolestes pusillus, the flour beetles Triobolium confusum and T. castaneum, the Indian meal moth, Plodia interpunctella, and the saw tooth grain beetle, Oxyzaephilus surinamensis (82). Fulton et al. (28)

cited the work of C. R. Phillips and R. K. Hoffman that indicated the amount of ETO required to kill all microorganisms is approximately 20 times as great as for insects tested. Since a high count of microorganisms is found more frequently in spices than are insects, fumigations are generally made for bacterial and fungal control and there is a kill of insects should they be present.

Method of Application, Dosage, and Use

Two ETO fumigant formulations are registered with EPA for fumigation of natural seasonings. Ingredients by weight of one formulation is 10% ETO plus 90% carbon dioxide (Reg. No. 10330-6) and the other is 12% ETO and 88% dichlorodifluoromethane (Reg. No. 10330-5). Fumigations are made under vacuum. The dosage and exposure as registered for both formulations is 15 lb of formulation per 1,000 cu. ft. and the length of exposure is 16 hours. The fumigation procedure generally followed is:

1. Load chamber with natural seasonings or spices (on pallets).
2. Seal chamber door.
3. Pull vacuum on chamber to 29 inches of mercury.
4. Allow 5 minutes for out-gassing.
5. Through volatilizer release formulation into chamber (expansion of gas will reduce vacuum in chamber to about 24 inches).
6. Hold for 16 hours of fumigation.

7. Break vacuum with atmospheric air.

8. Pull vacuum on chamber to 29 inches of mercury.

9. Break vacuum with atmospheric air.

10. Steps 8 and 9 complete one-air-wash, repeat steps for 2nd air-wash.

Another major use of ETO is the fumigation of black walnut meats. About 5 million lbs. of Eastern black walnut meats and 2 million lbs. of California black walnut meats are fumigated annually for control of microorganisms and insects of types similar to those found in the spice industry (21). The nut meats are fumigated under vacuum with a dosage of 3.5 lb. of actual ETO per 1,000 cu. ft., and an exposure period of about 16 hours. It is estimated that about 3,200 lbs. of actual ETO is used annually for fumigation of black walnut meats. Spices and nut meats are normally fumigated once and the fumigation is prior to packaging the condiments for commercial and retail distribution.

Ethylene oxide is registered for fumigation of furs, clothing and furniture. Based upon telephone contact with 3 furriers located in New York City, cold storage has replaced fumigation for protection of furs from damage by insects. Tyrone L. Vigo (88) of the USDA Textile and Clothing Research Laboratory through industry contacts did not find ETO was used for fumigation of clothing except in hospitals, nor did his contacts reveal any fumigation of domestically produced wool. Imported wool from Afghanistan has been fumigated for control of anthrax. None of three renovators of furniture in the City

of Richmond, Virginia, used ETO to fumigate reconditioned furniture, but instead applied heat or fumigated furniture and bedding with formaldehyde. This survey, although limited, indicates the amount of ETO used in the U. S. for fumigation of furs, clothing and furniture is negligible.

Fate of ETO in the Environment

Air:

At the end of the fumigation exposure period, ETO is released through an exhaust stack to the atmosphere. In the sterilization of disposable syringes, Jordy (38) measured the ETO concentration at the cap of a stack connected to a 15 cu.m. chamber charged with an ETO concentration of 1,330 g per cu.m. and held for an exposure period of 6 hours. During the first two minutes of emission ETO concentration at the cap of the exhaust stack was 500-600 g per cu.m. In the following four minutes the concentration decreased to the detection limit of 5 g per cu.m. In the subsequent air-wash, the maximum ETO concentration was 300 g per cu.m. and this concentration occurred only at the beginning of the emission from the first air-wash. Even at the beginning of ETO emission from the chamber, the concentration of more than 5 g per cu.m. could not be detected at a distance of 20 cm from the cap of the stack.

Importance of Maintaining ETO Uses for Stored Products

ETO is the only fumigant acceptable to industry for destruction of living microorganisms in spices, natural seasonings and black walnut meats. In the Federal Register of January

27, 1978 (43 FR 3800) submitted by HEW (84) statement is made "Many of these products cannot be sterilized by other means such as heat, filtration, radiation, or liquid chemical agents without degrading or otherwise damaging them: there are no other acceptable safe gaseous substitutes available". In this instance "these products" refers to drug products for human and veterinary use. Similar conditions prevail for protection of spices, natural seasonings, and black walnut meats.

Hall (35) states that fumigation of spices in a normally dry state will kill 98-100% of the bacteria, yeast, and mold spores. When spices are in an environment having favorable moisture and temperature conditions for bacterial growth the fumigation process showed its effect against spores by a kill of 73 to 100%. By ETO fumigation, spices and other ingredients used in food manufacture and processing may be freed from potentially harmful microorganisms contamination. This allows the food manufacturer and processors to exercise control for quality of their finished product.

Relative Effectiveness of Alternative Controls

Propylene oxide is the only registered chemical pesticide that may be used as an alternate for control of microorganisms in spices and nut meats. The formulation registered by EPA, No. 10330-10, is 8% propylene oxide plus 92% carbon dioxide, by weight. Except in the fumigation of packaged, dried prunes and glace fruits, it is applied in fumigation chambers not more than one time at a temperature not in excess of 125°F. The maximum period of fumigation shall not exceed 4 hours for

coca, processed nut meats (except peanuts, processed spices, and starch. For edible gums, the maximum duration shall be 24 hours, HEW (83). In a petition filed later by Union Carbide (March 12, 1976) the maximum period for fumigation was extended to 48 hours at 125°F. Fumigation schedules listed on current labels are:

Chamber pressure	Temperature	Dosage	Exposure
Atmospheric	100-125°F.	35 lb/1000ft^3	16-48 hr.
26" vacuum	100°F.	35 lb/1000ft^3	16-48 hr.
26" vacuum	125°F.	35 lb/1000ft^3	12-24 hr.

Propylene oxide is not as effective as ETO for control of microorganisms. Gammon and Kereluk (29) reported "prolonged periods of propylene oxide contact were required to obtain a greater than 90% reduction of the bacterial count". They predict propylene oxide, at best, is only half as effective as ETO. This is reflected in the approved fumigation schedules. In vacuum fumigation ETO dosage was 15 lbs. with exposure of 16 hrs whereas propylene oxide is 35 lbs for 12 to 48 hours depending upon temperature of the fumatorium.

Heat treatment of spices for control of microorganisms has been investigated by the industry but they found prolonged exposure caused an average loss of about 15% in spice strength. There was a lightening in color in some natural seasonings and a darkening in others (33).

Cost of Fumigant Formulations

These costs vary due to different charges made by formulators plus the transportation charges for delivery of the fumigant

from the formulator to the user. The following are estimated to be the average costs of formulation minus charge for transportation.

Fumigant formulation	Cost/pound
8% propylene oxide plus 92% carbon dioxide	$ 0.51
10% ETO plus 90% carbon dioxide	0.53
12% ETO plus 88% dichlorodifluoromethane	0.80

Summary

Spices are grown in the tropical regions of the Orient where they frequently become heavily contaminated with microorganisms. The spice manufacturers in the U.S. control potentially harmful bacterial, fungal or mold microbial organisms as well as insects by fumigating spices, natural seasonings and black walnut meats with ethylene oxide (ETO). The fumigations are made in chambers under vacuum. Control of bacteria, yeast, and mold spores is 98-100% when spices are in a dry state and 73-100% when spices are in an environment having favorable moisture and temperature conditions for growth of the micro-organisms.

Propylene oxide is the only registered chemical pesticide that may be used as an alternate for ETO fumigation. At best, it is only half as effective as ETO for control of micro-organisms. Heat will kill microorganisms, but prolonged exposures cause an average loss of about 15% in spice strength and there is a change in seasonings and black walnut meats

allow food manufacturers and processors a means for production of high quality products.

SUMMARY OF REPORT

On January 27, 1978, a notice of rebuttable presumption against registration and continued registration of pesticide products containing ethylene oxide (ETO) was published in the Federal Register (43 FR 3801). This action was taken because the Environmental Protection Agency (EPA) concluded that their evaluation of scientific studies and other information indicated that ETO exceeded the criteria for risk relating to mutagenicity and other chronic or delayed toxic effects, specifically reproductive effects.

General Usage and History

More than 50 years ago this chemical was found to be an effective fumigant for insect control. Later, ETO was found to be effective for the sterilization of foodstuffs. For a number of years, ETO has also been used as a sterilant for certain human and veterinary drug products and for the sterilization of medical and laboratory equipment. ETO is used extensively in medical and other laboratory facilities for the sterilization of equipment and supplies that are heat sensitive.

Commercial production of ETO in the United States was reported to be over five billion pounds in 1977. The chemical is used primarily as an intermediate in the production of ethylene glycol antifreeze and coolant, and for derivatives of nonionic surfactants, glycol ethers, and ethanolamines. The amount of ETO used as fumigants or sterilants is estimated to be less than one percent of total production. There are 39 Federally registered pesticide products containing ETO and three ETO products

registered in states as fumigants or sterilants.

The current U. S. Occupational Safety and Health Administration Standard for exposure to ETO is 50 parts per million (ppm) in air, as a time weighted average (TWA) concentration for an 8-hour exposure. There are EPA-established tolerances of 50 ppm (40 CFR 180.151) on certain stored food products.

Importance of ETO to Agriculture

The benefits derived from its use as a fumigant and sterilant and the lack of adequate substitutes show that ETO is essential to Agriculture and its related industry. ETO is used in Agriculture in the following areas:

Apiculture

Maintaining the beekeeping industry in a healthy condition is essential to our agricultural economy. More than 90 crops, valued in excess of $8 billion are benefitted by bee pollination. Inadequate pollination can result not only in reduced yields but also in delayed yields and a high percentage of culls or inferior fruits. Fumigation with ETO is becoming an invaluable tool for the prevention and control of bee diseases and thus aids in the maintenance of healthy bee colonies.

Contaminated beekeeping equipment is the principal reservoir for bee disease agents. ETO is used to sterilize and allow recycling of contaminated equipment. Its primary use in beekeeping is to recycle equipment from known American foulbrood (AFB) diseased colonies. ETO is also effective against European foulbrood (EFB), chalkbrood, and nosema diseases. Tests indicate that colonies placed in ETO fumigated hives develop larger

populations due to controlling unidentified diseases of honey bees. In addition, pests such as the greater wax moth are also controlled by ETO.

American foulbrood is the most widespread and the most destructive brood disease of honey bees. AFB is caused by a spore-forming bacterium and the disease spreads rapidly when bee equipment contaminated with large numbers of the AFB spores are transferred to healthy hives. Colonies which have AFB die out if the disease is not controlled. The destruction of AFB diseased colonies is included in most State apiary laws and regulations.

European foulbrood is another bacterial disease that is spread in the same manner as AFB disease. Colonies can be weakened or die if EFB is not controlled.

Chalkbrood is a fungal disease that normally does not destroy a colony; however, it can prevent normal population levels when the disease is serious. The spores remain viable for years and, as with AFB and EFB contaminated bee equipment, are the chief source of reinfection.

Nosema disease is a major disease of adult honey bees and can cause extensive losses. The causative organism is a protozoan and is also spread through the use of contaminated equipment.

At the present time five states have a section 24(c) registration under the Federal Insecticide, Fungicide and Rodenticide Act for using ETO. Seven states and the USDA Bioenvironmental Bee Laboratory are using ETO on an experimental basis. It is estimated that 1,500 pounds AI are used annually.

The Food and Drug Administration has approved labeling for terramycin and fumagillin as aids in the control of bee diseases. Terramycin can control AFB and EFB but it is not a practical alternative since the antibiotic will not kill the casual organisms. Disease development is only controlled while the antibiotic is present in the larval food. Some states permit the use of antibiotics while others require the destruction of the colonies with AFB diseased colonies by burning or decontamination of the hive parts and equipment. Some areas restrict or prohibit open burning. Fumagillin has been used with some success in controlling nosema disease. Nosema disease can be decontaminated by exposure to a temperature of 120°F. for 24 hours or by acetic acid fumigation. There is no alternative treatment presently available for the control of chalkbrood.

There is concern about the use of antibiotics that may lead to the development of strains of drug resistant bacteria making control more difficult, and the possibility of contamination of market honey with antibiotic residues.

High Containment Research Laboratories in Agriculture

The USDA maintains high-containment laboratories devoted to research and diagnosis of domestic and exotic (foreign) plant and animal disease pathogens. ETO is used in the many decontamination/sterilization operations that must be followed to protect laboratory workers and susceptible plant and animal populations.

Three USDA facilities - Plum Island Animal Disease Center, Greenport, New York; National Animal Disease Center, Ames, Iowa;

and the Plant Disease Research Laboratory, Frederick, Maryland, have high-containment laboratories. The types of animal and plant diseases which the scientists are working with require many safeguards to maximize the containment of the pathogens. Some safeguards include: maintenance of increasing degrees of reduced air pressure from areas of lesser to greater contamination; high volume-single pass air flow, with all air filtered before discharge; self-contained waste water sterilization facilities; incinerators; and the wearing of special clothing and decontaminating showers before exiting laboratories.

These laboratories are responsible for maintaining diagnostic capabilities for a variety of domestic and foreign pathogens as well as conducting basic and applied research relative to these agents. It is the policy of the USDA to take appropriate decontamination/sterilization measures to ensure that there is no possibility of introducing animal and plant disease organisms into the environment of the United States. Research is currently being conducted on such animal diseases as foot and mouth disease, African swine fever, influenza, swine vesicular disease, rinderpest, and sheep pox. Plant disease research includes various downy mildew and rust diseases of corn and sorghum and the soybean rust disease. Since the animal populations and various varieties of plants are totally susceptible to these pathogens, the introduction of these agents would result in a very rapid spread of the disease. For example, it has been estimated that a major outbreak of the foot and mouth disease virus could cost $12 billion over a 15-year period.

ETO is frequently applied to decontaminate and sterilize laboratory equipment between uses. There are many laboratory items which are delicate in their make-up or the material itself is heat labile and the exposure to heat could destroy or severely damage the items. Some equipment and supplies are moisture sensitive and cannot be sterilized by chemical solutions.

Every item removed from the high containment laboratories to other locations for use, repair, or modification must be decontaminated. Training films, electronic, electrical, optical, and other equipment must be sterilized with ETO. Training films prepared in these animal laboratories are utilized at many universities as part of the training for early diagnosis of exotic animal diseases.

ETO is the only sterilant that can be used safely and effectively without damage to many items. ETO is highly diffusive and will penetrate areas not reached by liquids or steam. There are many laboratory items that are essential tools utilized in every phase of laboratory research. If ETO registered uses were cancelled, a significant number of these items would no longer be available. It would not be economically feasible for such expensive equipment to be purchased on a one-use-and-discard basis.

All ETO sterilizer chambers are operated as closed systems. Double-ended sterilizers are available which allow for the removal of certain materials from the interior (contaminated) to the exterior of the laboratory. Sterilizers are not used every day. Average use is 2-4 times per week. The 12% ETO-88%

dichlorodifluoromethane formulation (12-88 mixture) is used. The annual usage of the mixture at these laboratories is estimated to be 4,340 pounds.

Steam sterilization and dry heat are effective sterilants but are not acceptable for many heat or moisture sensitive materials. A wide variety of alternative decontamination agents have been investigated, but none have met all the criteria of efficacy, reliability, and safety. There is no known substitute to ETO for many laboratory sterilizations.

Plant Protection and Quarantine Programs

ETO is an important fumigant/sterilant recommended by the U. S. Department of Agriculture as a quarantine fumigant against snail contaminated cargo and as a sterilant to control certain plant disease organisms.

To prevent the entry, establishment, and spread of snails of quarantine significance, imported cargoes which are infested are held under quarantine until decontamination can be accomplished. Methyl bromide and ethylene oxide are the two fumigants recommended by the USDA for treating the contaminated cargo. Where methyl bromide cannot be used due to its deleterious effects on certain materials or its development of objectionable odors, fumigation with a 10% ethylene oxide and 90% carbon dioxide mixture can be effectively used.

Most snail interceptions at ports of entry have been found on retrograde military materials. The location of the U. S. Department of Defense installations throughout the world has exposed weapon systems and other material to native snails at

overseas installations. If ETO was cancelled for quarantine fumigation purposes, the only alternative would be to refuse entry to infested shipments into the United States or fumigate with methyl bromide. The damage that might occur from the use of methyl bromide could result in the destruction of valuable equipment and materials.

Quarantine fumigations are conducted by commercial pest control operators under USDA supervision and according to schedules and procedures listed in the Plant Protection and Quarantine Programs Treatment Manual (85). Fumigation of snail contaminated cargo is normally conducted under tarpaulin because of the quantity and dimensions of the infested cargo. Tarpaulin fumigation allows very large amounts of intransit cargo to be treated expeditiously and reduces the risk of pest spread by fumigating near the cargo discharge area. In calendar year 1977, 64 tarpaulin fumigations were conducted, using 7,185 pounds of the 1:9 fumigant mixture. Fumigation dosages range from 20-27½ lbs. of the mixture per 1000 ft^3. Chamber fumigations are fewer than five treatments per year.

Recent investigations (61) indicate that ETO could be successfully used as a fumigant for termites and wood boring insects. An ETO fumigation schedule has been developed for tick contaminated material. Records indicate that this treatment has not been used during the past three years.

The USDA has tested (62) all commonly used fumigants against land snails. Methyl bromide, hydrocyanic acid, and ethylene oxide have been found to be the only effective chemicals.

Methyl bromide cannot be used on all snail contaminated cargo. Hydrocyanic acid cannot be used safely under tarpaulin fumigations and is not readily available. Exposure of the snails to low temperatures is an effective control but refrigeration facilities to handle large shipments are not available and the necessary safeguards to prevent pest escape, if they were available, would be extensive and not practical.

Sterilization with steam, dry heat, and ETO are effective quarantine type treatments for the control of plant disease organisms. Because of the danger of the introduction of foreign plant disease, certain imports are subject to a sterilization treatment as a condition of entry. For those materials which would be damaged by a heat treatment ETO sterilization is authorized. Treatments are conducted in vacuum chambers at a dosage rate of 25 lbs. of ETO per 1000 ft^3 for 24 hours. For these imports, fewer than 25 sterilization treatments are conducted each year.

Stored Products

Spices and natural seasonings are carriers of bacteria, molds, and yeasts in a dormant state. ETO is widely employed in the spice industry to control pathogenic organisms and to reduce other microbial populations. In addition to the direct health benefits, the reduction of the microorganisms assures the adequacy of existing perservation measures for canned and frozen foods, eliminates the need for increased processing of many foods, and enhances their shelf-life.

Over 141 million pounds of spices with a Custom value of 132 million dollars were imported into the United States in 1977. Spices are imported from many parts of the world and become contaminated with microorganisms during the growing period and handling after harvesting. The most frequently used spices, black and white pepper, are heavily contaminated. Over 100 million pounds of spices were treated with ETO in 1977 to free them from harmful microorganisms. The quantity of ETO employed for this use was approximately 750,000 pounds.

A number of stored product insects are also found with spices. The ETO fumigation schedules used for the microorganisms also kills these insects thus avoiding a separate fumigation for insect control.

Another major use of ETO is the fumigation of black walnut meats. About seven million pounds are treated for the control of microorganisms and stored product insects. It is estimated that 3,200 pounds of ETO is used annually for these fumigations.

Propylene oxide is the only registered chemical that may be used as an alternative to ETO. However its higher boiling point makes it more difficult to remove after treatment. Further disadvantages include its lack of effectiveness on bacteria as compared to ETO, and it is not approved for use on whole spices. Heat treatment was investigated about 30 years ago and the prolonged exposure period to heat caused a loss of 15% in spice strength, a lightening in color in some natural seasonings, and a darkening in others.

Exposure Conditions

A review of ETO fumigation/sterilization procedures shows that the size of the worker population that may be exposed to ETO is small and the duration of exposure, if any, is short. Exposure potentials are occasional in which most of an individual's working day is without possibility of exposure. The likelihood for exposure is greatest during the application of ETO, leakage from the chamber or enclosure, loading and unloading of the treated materials, and during the aeration period.

In normal usage of ETO, in gas tight chambers, the chance of the operator being exposed to the gas is minimal. Use of repeated vacuum-air introductions to "air wash" and remove the sterilant to the outside atmosphere following the treatment period can reduce the potential hazard exposure to the operator. An adequately vented-aerated environment around the fumigation/sterilization site plus commonly recognized safety precautions minimize worker exposure. At fumigation sites located outdoors the potential exposure conditions can be minimized by the use of respiratory protective equipment as recommended by the manufacturer. Standards for the use of respiratory protection equipment will be published by the American National Standards Institute, in the near future stating the type of equipment and at what stage in the operations it must be used. These standards will be adopted by OSHA as acceptable procedures.

CONCLUSIONS AND RECOMMENDATIONS

The honey bee industry needs ETO for treating hive equipment for American foulbrood and other bee diseases. There is no suitable, registered alternative chemical available. ETO fumigations can allow for recycling disease contaminated equipment. Without the use of this chemical, the only alternative is to destroy the equipment by burning.

The loss of ETO as a sterilant and decontaminant in high containment laboratories would seriously compromise biological safety standards or preclude research in those areas of study. There exists no known substitute for ETO for many specific sterilization and decontamination procedures. Having to use alternative sterilants would result in the loss by destruction of a wide variety of tools, instruments, and equipment which are either recycled for within-laboratory use or which need to be removed from these laboratories. The continued use of ETO in high containment laboratories is essential. The biological containment mandates governing the operations of the facilities cannot be compromised.

The loss of ETO as a fumigant would deny the USDA quarantine programs the use of an effective chemical to treat snail contaminated imports. The only alternative fumigant is methyl bromide. Methyl bromide can cause damage to certain materials and cause the development of objectionable odors. The damage which could occur with methyl bromide could result in the destruction of valuable equipment and materials.

The effectiveness of ETO as a sterilant is well established. Dry heat or steam sterilization which are the only effective alternatives cannot be used on all intended imports.

Without the continued use of ETO, pest contaminated imports would have to be refused or denied entry into the United States for lack of an effective fumigant/sterilant treatment.

ETO fumigation is the only effective means of treating spices and black walnut meats to eliminate pathogens in food prepared and consumed by the general public. There are no approved, effective alternatives to ETO to achieve comparable microbial reduction or protection against pathogenic organisms on spices. Propylene oxide which is the only feasible alternative is only one-half as effective as ETO and it is not approved for use on whole spices. Without an effective fumigation treatment, spices could potentially contain sufficient levels of microorganisms to require destruction or could fail the microbiological requirements for industrial use.

The importance of retaining ETO as a fumigant/sterilant in Agriculture and related industries is essential. A ban of this chemical would have various substantial adverse repercussions. The continued use is highly desirable. Alternative chemicals or other processes have, in themselves, serious limitations or health hazards.

Unnecessary exposure of workers can be minimized by: training the fumigator/sterilizer operators; proper venting of equipment, working area, sterilized items, and the storage area; and the improvement of operating techniques and design

of the treatment facilities.

A number of techniques are available for the analytical determination of low concentrations of ETO in air. Cooperative monitoring by EPA/USDA of the ETO concentrations at selected fumigation/sterilization sites could be conducted to determine exposure levels.

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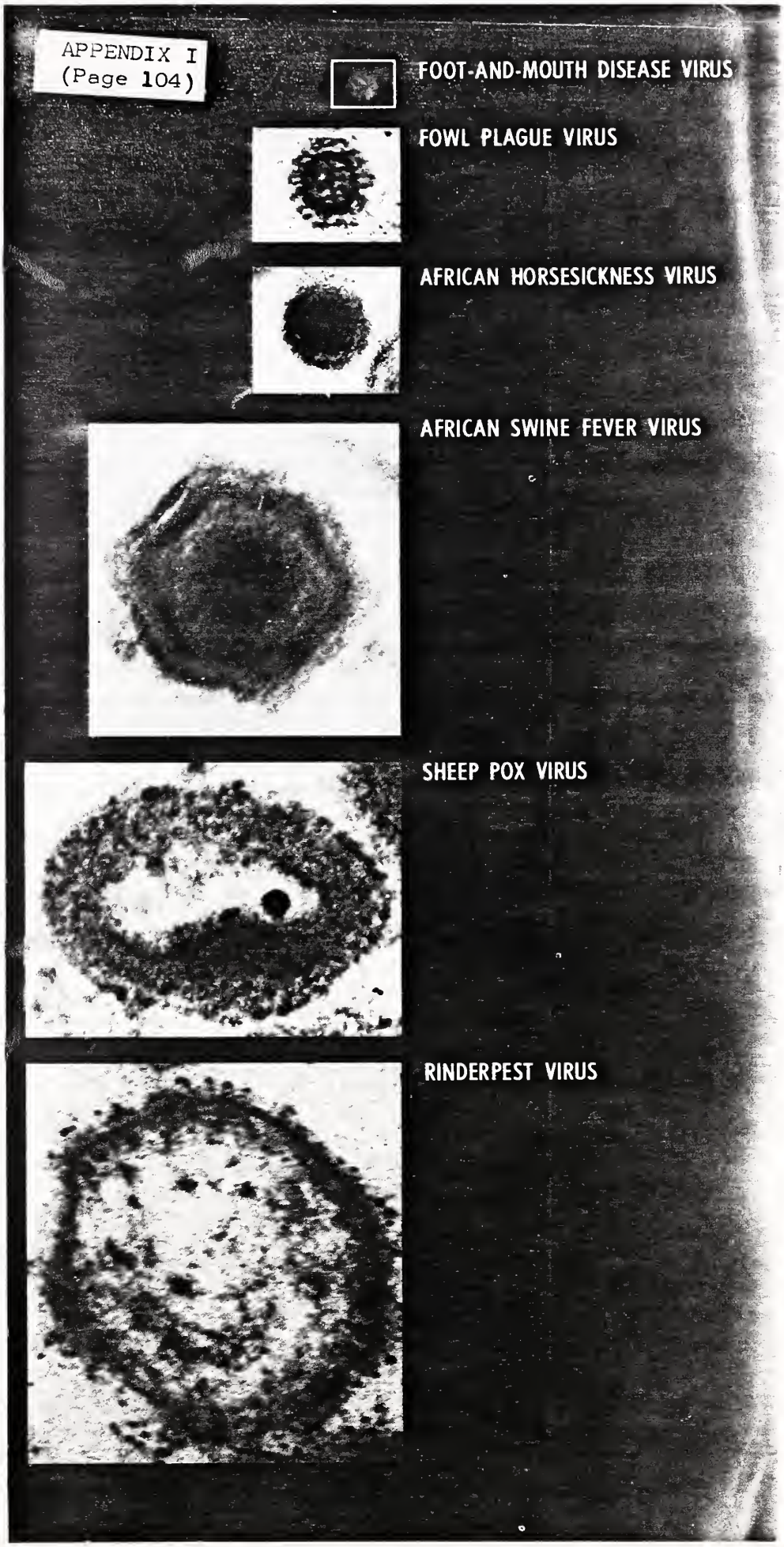
THE PLUM ISLAND ANIMAL DISEASE CENTER

Research on Foreign Diseases of Animals



U.S. DEPARTMENT
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CONTENTS

Note: The pictures of the virus particles on the cover are electron micrographs taken at Plum Island. They are magnified c. 175,000 times.

This publication supersedes Miscellaneous Publication 931, "The Plum Island Animal Disease Laboratory."

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THE PLUM ISLAND ANIMAL DISEASE CENTER

Research on Foreign Diseases of Animals

The Plum Island Animal Disease Center is the only research facility in the United States devoted to the study of contagious foreign diseases of animals. It is located on an island east of Long Island, N.Y., and is operated by the Agricultural Research Service, U.S. Department of Agriculture.[1]

The Department is responsible for:

- Developing capability of diagnosis of animal diseases that do not exist in the United States.
- Conducting basic and applied research on foreign animal diseases and their causative organisms.
- Developing adequate procedures so that foreign, domesticated, and wild animals, and semen, meat, and other animal products may be imported safely.

The Center conducts fundamental research to develop the necessary knowledge that enables the Department to carry out these responsibilities. The main objective is to prevent the introduction of diseases that could result in high death tolls or serious economic losses in our susceptible livestock population.

LOCATION AND HISTORY

Plum Island is located 110 miles from New York City, about 10 miles from Connecticut, and about 1-1/4 miles off the northeastern end of Long Island, N.Y. The island contains about 800 acres and is about 3 miles long and a mile wide in the western, or widest part. It is reached by boats operated by the Center from Orient Point, Long Island, where the Center has a harbor, an office building, and storage facilities for incoming supplies.

Plum Island was named by early explorers who observed beach plums growing along the shores. In 1659 the ruling Indian chief of Long Island sold Plum Island to the first European owner, Samuel Wyllis, for "a coat, a barrel of biscuits, and 100 muxes[2] or fishhooks."

The U.S. Government bought the island in the 1890's and established Fort Terry, a coast artillery post. The island was assigned to the Army Chemical Corps after World War II. On July 1, 1954, all of Plum Island, except for a U.S. Coast Guard lighthouse, was formally transferred to the U.S. Department of Agriculture for the establishment of a Center for the study of exotic diseases of domestic animals.

Preliminary studies were started in 1954. When additional laboratory facilities became available in 1956 the Center's research was expanded into a broad program covering many foreign animal diseases.

THE CENTER'S MISSION

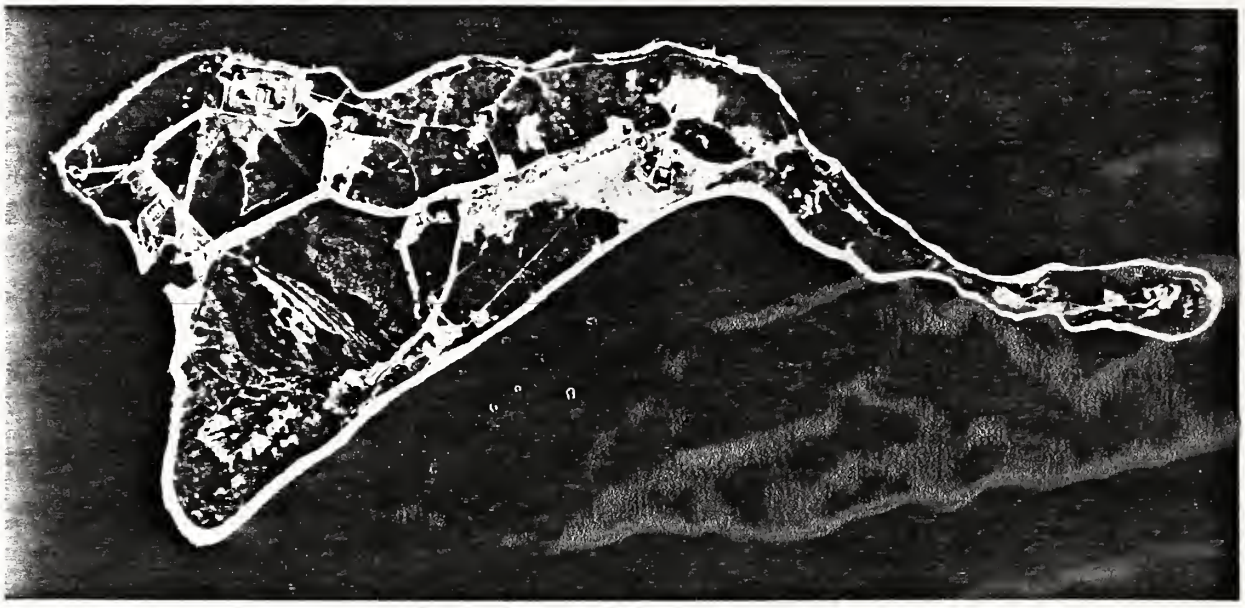
The mission of the Center is to perform the following basic and applied *research* and *service* work on the various contagious foreign diseases of animals, with primary emphasis on foot-and-mouth disease.

Research

- Basic research on viral structure, pathogenesis of the disease, antigen-antibody

[1] Mailing address: P.O. Box 848, Greenport, Long Island, N.Y. 11944.

[2] Muxes are small drills the Indians used to make holes in wampum.



PN-3648

Aerial view of Plum Island.

reactions, and host and disease-agent relationships.

- Applied research on virus survival in animals and animal products, methods of virus inactivation, and development of vaccines and other control measures.

Service

- Diagnosis of disease by laboratory tests on specimens from animals in suspected field outbreaks.
- Tests for infectious agents in semen or specimens from live animals prior to importation.
- Assessment of hazards from imported products.
- Production of diagnostic materials for other laboratories.
- Training of U.S. and foreign personnel.
- Technical assistance to foreign countries to lower disease rates and thus reduce hazards to the United States.

Technical support to other Federal agencies includes diagnostic services, specialized studies on animal products, and development and evaluation of new techniques.

Emergency services are performed as required for diagnosing foreign animal diseases. When materials from disease outbreaks of suspected foreign origin are submitted to control agencies, studies are conducted to determine whether a foreign animal disease is involved.

Tests are made on throat fluids, serum, and semen to determine whether animals or semen for importation may be infected with foot-and-mouth disease virus.

Training courses are given so that diagnosticians in the field will become more familiar with the foreign diseases of animals, which they may have to recognize and investigate if outbreaks occur.

Specialized studies on animal products such as meat and semen are made to assist control agencies in deciding whether certain animal products should be admitted from foreign countries and what may be done to render them safe from a disease standpoint. Certain foreign biological products require a similar safety evaluation. If this service had been available in 1908, an outbreak of foot-and-mouth disease in this country might have been averted. The outbreak was traced to contaminated imported smallpox vaccine, which was propagated in calves.

New disinfectants and sterilization techniques also are evaluated to assist the work of control agencies in dealing with foreign animal diseases.

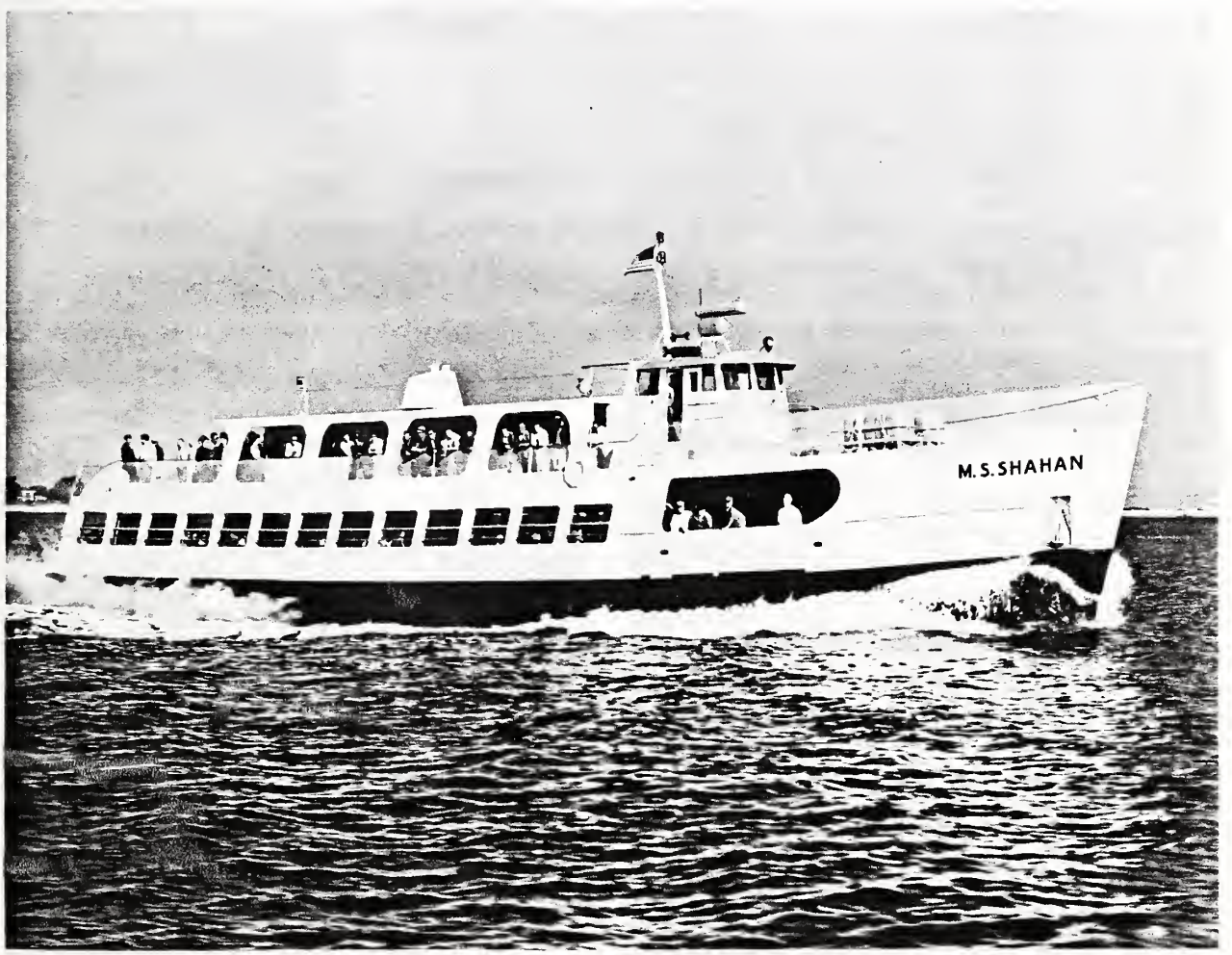
SAFETY PRECAUTIONS

Because of the Center's location as well as its special facilities, devastating foreign animal diseases can be studied without endangering livestock on the mainland. Congress provided this protection for U.S. livestock by specifying that the Center be on an island entirely under Federal control and be separated from the mainland by deep navigable water.

Rigid safety regulations were also devised to prevent the escape of highly communicable disease- causing agents from one research area to another and the accidental introduction of extraneous domestic disease agents, which would complicate the studies.

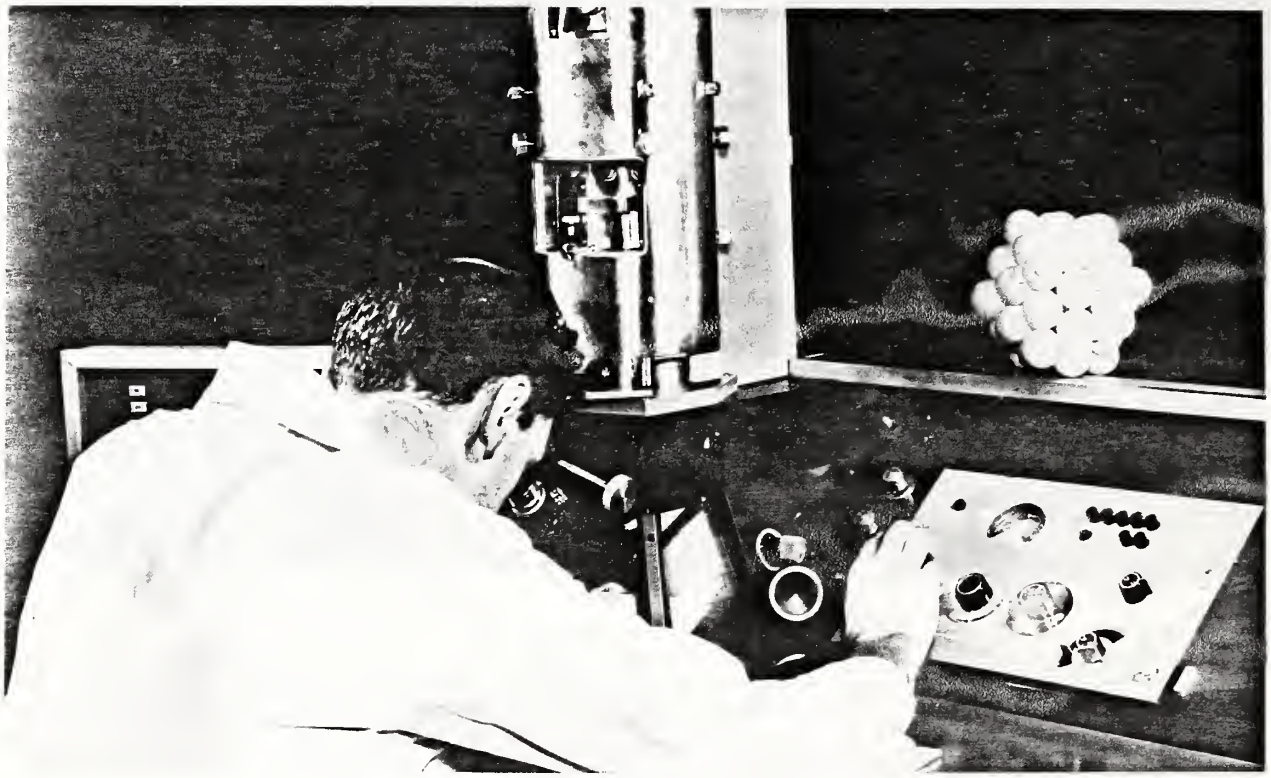
The Federal Government controls all movement to, from, and on the island. Only authorized persons are permitted entry to the island; entrance to the laboratory buildings and animal quarantine areas is restricted. All Center personnel are prohibited from contact with susceptible species of animals, or premises where such animals are held, for specified periods of time after leaving the island (7 days for persons working in the laboratories and 3 days for other personnel).

The two main laboratory buildings on Plum Island were specifically designed for research on highly communicable diseases and are considered among the safest in the world for work on animal viruses. All entrances and exits



PN-3649

The "M. S. Shahan" is used to transport employees to and from Plum Island.



PN-3650

A scientist uses the electron microscope.

PN-3651

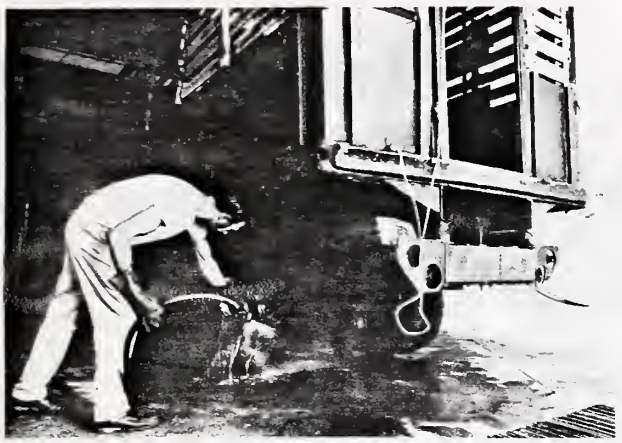
A technician inoculates chicken egg embryos to detect virus.

for personnel, animals, and supplies are strictly controlled. Persons must change to laboratory clothing upon entering the building. Upon leaving, they must take a decontaminating shower before putting on their own clothing.

Exhaust air from these buildings is decontaminated through a system of filters, and all liquid

PN-3652

Foreign scientists observe a rapid diagnostic test for African swine fever.



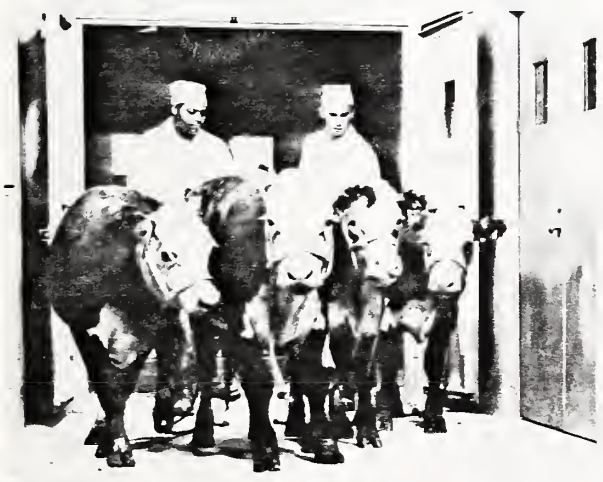
PN-3653
A technician disinfects a truck that must be removed from Plum Island.

PN-3655
Genetically stable laboratory mice are raised especially for Plum Island research.

wastes are sterilized by heat before being discharged. Solid wastes, including animal carcasses, are destroyed by incineration within the research buildings.

SUPPORT ACTIVITIES

Because of its isolation, the Center maintains all services needed to support its research. It employs an administrative staff, engineers, animal caretakers, maintenance men, a safety staff, guards, firemen, and other workers, in addition to scientists and laboratory technicians.



PN-3654
Cattle are introduced into the laboratory through air locks.

The Office of the Director provides overall guidance and management for the research and supporting groups.

Administrative Management Services is responsible for personnel work, purchase and delivery of supplies, and operation of food, photographic, and duplicating services.

The Safety Office places major emphasis on preventing the escape of disease agents from the Center. Other programs include industrial and fire safety, first aid, and plant security.

Animal Supply maintains colonies of disease-free guinea pigs, mice, and other small laboratory species to supply research sections. All large experimental animals, such as cattle, sheep, goats, and swine, brought to the island are inspected. Quarantines are placed on all animals until they are needed for research. Animal Supply also provides whole blood, tissues, and serum from normal animals for use in diagnostic tests and tissue cultures.

Laboratory Services provide tissue cultures and prepare media and sterile equipment for use in the laboratory. This group also operates a laundry and a glassware-washing service.

The Library acquires and makes available scientific books, journals, and reports necessary for animal disease research. It provides reference and reprint services.

Engineering and Plant Management is responsible for the maintenance, repair and construction of laboratory facilities and equipment, utility plants and systems, harbors and docks, pavements and grounds and various support structures. It also provides all utility

PN-3656

A supervisory data center is used to control and monitor equipment at Plum Island.

support services such as electrical power, heating, water, sewage decontamination and processing plus marine and automotive transportation.

DISEASES STUDIED

The contagious foreign animal diseases studied and diagnosed in the Plum Island Center and the principal domestic animals they infect include—

- Foot-and-mouth disease—cattle, hogs, sheep, goats.
- Rinderpest—cattle.
- Teschen disease—hogs.
- African swine fever—hogs.
- Fowl plague—poultry.
- African horsesickness—horses, mules, asses.
- Asiatic Newcastle disease—poultry.
- Lumpy skin disease—cattle.
- Ephemeral fever—cattle.
- Duck virus enteritis—ducks.
- Vesicular exanthema of swine—swine.
- Louping ill—sheep.
- Ovine and caprine pox—sheep, goats.
- Nairobi sheep disease—sheep.
- Rift valley fever—sheep, cattle, goats.
- Bovine herpes mammillitis—cattle.
- Exotic vesicular stomatitis—cattle, sheep, goats, swine, horses.
- Swine vesicular disease—swine.
- Borna disease—horses.
- Peste des petits ruminants—sheep, goats.
- Equine encephalosis—horses.
- Contagious bovine pleuropneumonia—cattle.
- Contagious caprine pleuropneumonia—goats, sheep.
- Contagious agalactia—sheep, goats.
- East Coast fever—cattle.

The diseases listed are caused by viruses except for the last four. Contagious bovine and

caprine pleuropneumonias and contagious agalactia are caused by mycoplasmas and East Coast fever by a blood parasite (hematozoan). Some of the diseases affect wild animals and birds in addition to domestic animals. About 70 percent of the research and service work is devoted to foot-and-mouth disease because of its great economic importance. Techniques and materials are being developed for rapid diagnosis of this and the other foreign diseases in the event of outbreaks here.

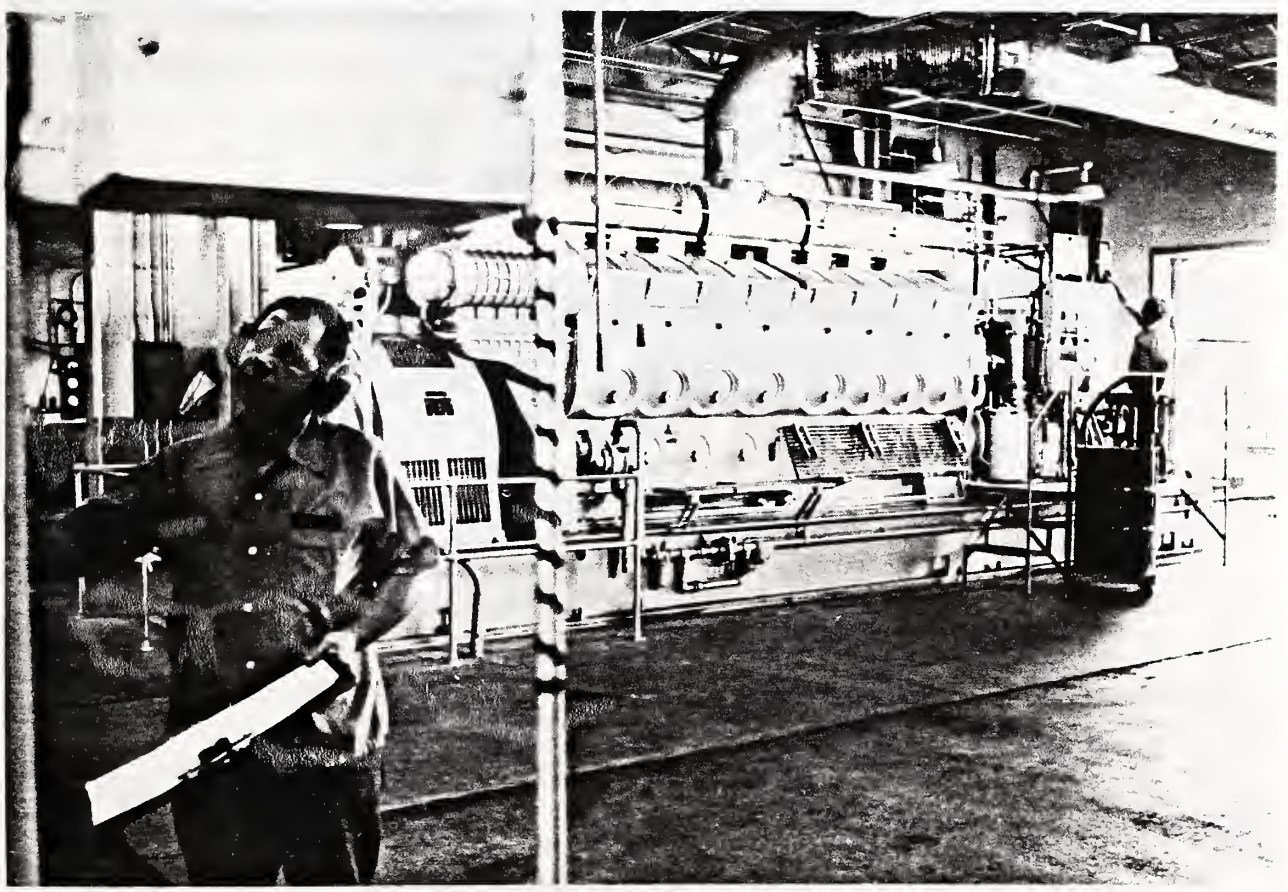
The Center's program is flexible enough to allow the study of additional disease problems when necessary. An outbreak of duck virus enteritis (duck plague), in the Long Island duck industry, necessitated a comprehensive study of this disease. When hog cholera is officially eradicated from this country, it also will be added to the list of foreign diseases to be studied and diagnosed.

GENERAL AREAS OF RESEARCH

Research at the Center has been divided into five general areas. These areas broadly correspond to the work of five different research groups, or disciplines. The general areas of research are as follows.

Viruses
(1) Biophysics
(2) Protein coat structure
(3) Nucleic acid structure
(4) Synthesis
(5) Antiviral agents

Vaccines
(1) Virus production
(2) Inactivants
(3) Adjuvants
(4) Safety and serologic testing
(5) Immunity challenge



PN-3657

Standby electric generators are always ready in case power from Long Island is cut off.

Cell Cultures

(1) Kinds of cells
(2) Cell nutrients
(3) Virus yield
(4) Viral changes
(5) Interference

Control Measures

(1) Virus persistence in animals, products, and environment
(2) Carrier studies
(3) Pathology and diagnosis
(4) Epizootiology and host range
(5) Disinfection

Diagnostic Tests

(1) Complement fixation
(2) Neutralization
(3) Agar gel diffusion
(4) Fluorescent antibody
(5) Agglutination

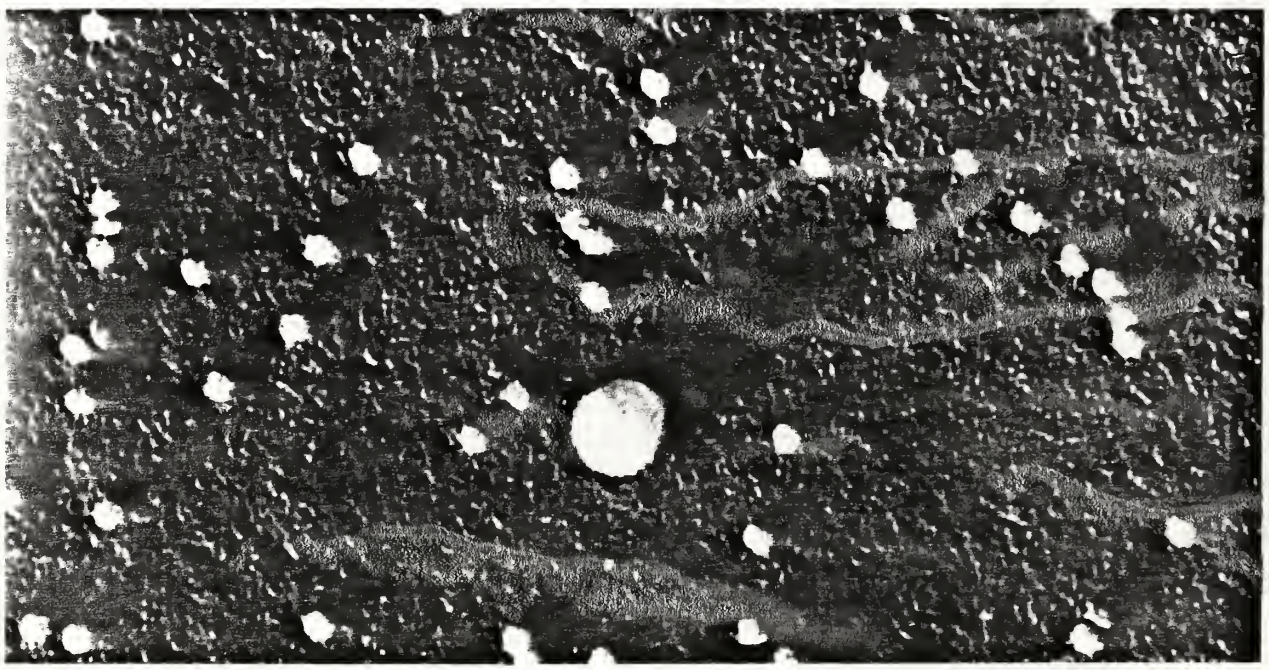
RESEARCH DISCIPLINES

The five research .disciplines are biochemical and biophysical, immunological, cytological, microbiological, and diagnostic investigations. These research disciplines are composed of veterinarians, virologists, bacteriologists, pathologists, chemists, physicists, and their technical assistants. Working alone or as teams, the scientists and their assistants are assigned to one of the five research disciplines.

Biochemical and Physical

Scientists in biochemical and physical investigations are concerned with problems in molecular biology. These scientists produce milligram quantities of foot-and-mouth disease virus in cultures of baby hamster kidney cells and purify the virus for use in biochemical and immunological studies. They examine animal virus particles for their size and shape by electronmicroscopy and for their chemical properties, including resistance to mechanical treatment, pH changes, thermal changes, and variations in ionic strength. They determine the effects of enzymes and chemicals as purifying agents and inactivants; study viruses intact and broken down into their protein and infectious nucleic acid subunits; determine diffusion, electrophoretic, and sedimentation rates of viruses and their subparticles.

The scientists in this group also investigate the correlations of physiochemical properties with infectivity, immunogenicity, and antibody-



PN-3658

An electron micrograph shows foot-and-mouth disease virus particles.

PN-3659

A technician operates a shadow casting instrument so that virus preparations can be viewed as three-dimensional objects.

antigen relationships. They study the mechanism of virus synthesis and its inhibition in tissue culture and cell-free systems by biological and chemical methods, using radiobiological tracers.

Immunological

Scientists in immunological investigations check the response of animals infected with or vaccinated against disease agents and they conduct research on the antibodies that protect against disease. Serum from these animals is separated into elemental components, and these are then analyzed by serological, chemical, and animal-testing techniques. They also study antigens or viruses that cause the disease.

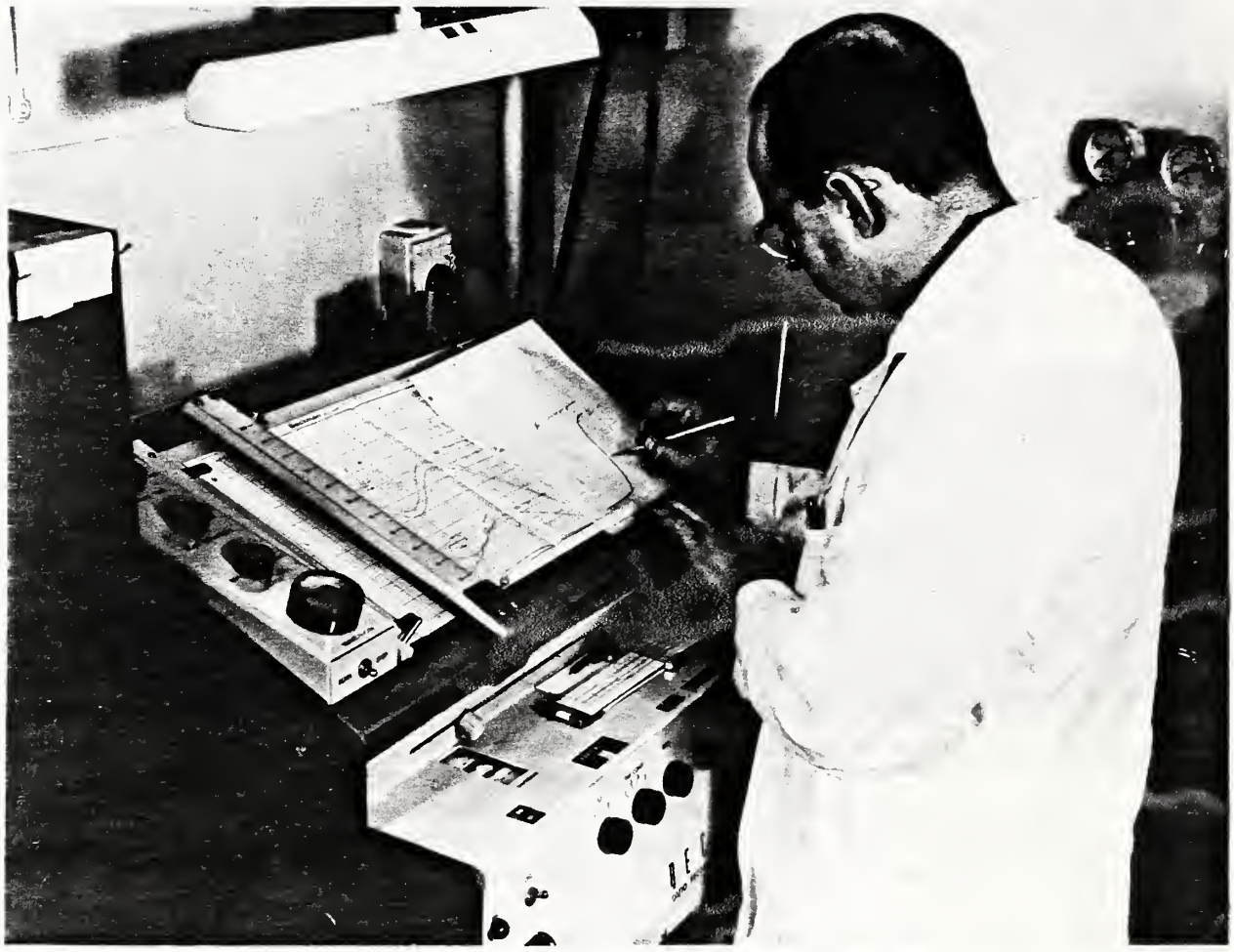
Another function of this group is the development and testing of vaccines appropriate for possible use if established disease-eradication procedures should fail to control invasions of foreign diseases. These studies include chemical inactivation of virus and development of critical tests to determine the safety and potency of vaccines produced. Such research requires the vaccination of many animals and the study of their immunity by serological and challenge methods.

Cytological

Scientists in cytological investigations study viral inhibitory substances, changes in viruses caused by environmental conditions, and growth of viruses in cell cultures. They have found that treatment of cells with certain chemicals stimulates the production of inhibitory substances effective to a limited extent against foot-and-mouth disease virus. They are investigating the possibility that these substances may be useful in the prevention of the disease.

Other scientists are attempting to change the foot-and-mouth disease virus by various procedures so that it no longer produces disease and thus might be used as a live attenuated virus vaccine.

Work in the cytological group also includes investigations on the viral susceptibility of various types of cell cultures and factors that



PN-3660

A scientist studies the results of foot-and-mouth disease virus on a ratio recording instrument.

affect their susceptibility. These studies are being done to obtain highly susceptible cultures for diagnosis of viral diseases and for other work involving assay or production of viruses.

Microbiological

Scientists in microbiological investigations study the susceptibility of various species of animals to virus diseases, explore ways in which the diseases spread, and determine in what organs and tissues the virus may be found. They study the factors that result in animals becoming virus carriers. They also trace the survival of viruses in meat, blood, semen, and other animal products. From the results of these studies, the U.S. Department of Agriculture is able to assess the hazards of importing live animals and animal materials from foreign countries in which dangerous diseases exist.

Scientists also study the effects of chemical and physical environments on viruses and thus contribute to knowledge regarding methods of virus inactivation, disinfection of contaminated materials and premises, and survival of viruses under various conditions. Such information is vital in preventing disease and eradicating outbreaks.

Diagnostic

When USDA veterinary diagnosticians in the field observe animals showing clinical signs suspicious of foreign animal disease, they collect samples and submit them to Plum Island. At

Plum Island, the staff of diagnostic investigations conducts various serological tests, virus isolations, animal inoculations, and pathological studies to determine if the samples were positive or negative for a foreign animal disease.

Considerable work is required to have in readiness all of the virus strains, antiserums, and cell cultures needed for the various diagnostic tests. In addition, research is conducted on various aspects of foreign diseases of animals other than foot-and-mouth disease.

RESEARCH HIGHLIGHTS

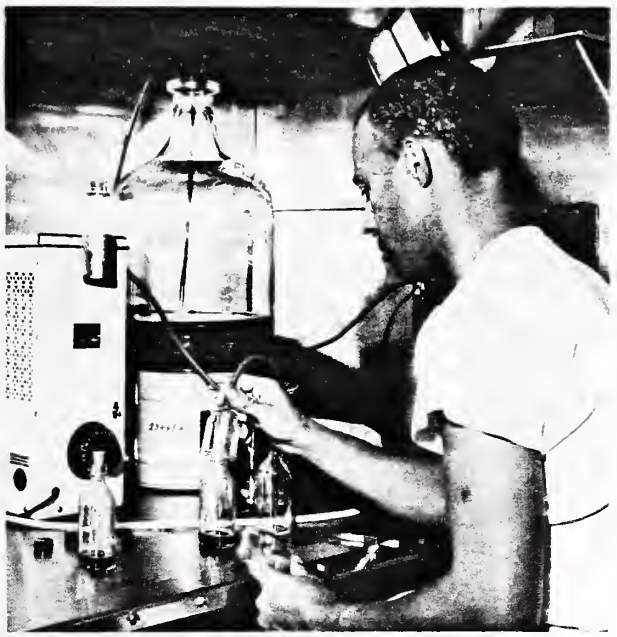
Foot-and-Mouth Disease (FMD)

Viewed foot-and-mouth disease virus (FMDV) in the electron microscope as a spherical particle 23 nanometers (about one-millionth inch) in diameter with 32 capsomeres on its surface.

Established that FMDV ribonucleic acid (RNA) can be encapsidated in a bovine enterovirus (BEV) protein coat and that such viral particles have biophysical properties of BEV but can produce FMDV in further growth cycles.

Developed an *in vitro* system for the study of the synthesis of FMDV-RNA.

Showed that FMDV-infected cells contain an enzyme, RNA polymerase, which is induced by the virus, is necessary for viral replication, but is not found in normal cells.



PN-3662

A technician dispenses kidney cells into tissue-culture bottles.

PN-3661

A technician cultures swine blood to determine sterility.

Showed that the virus replication activity of RNA polymerase is inhibited by antibodies produced in FMD-infected animals. Thus, RNA polymerase may be identical to a virus-infection-associated antigen (VIA).

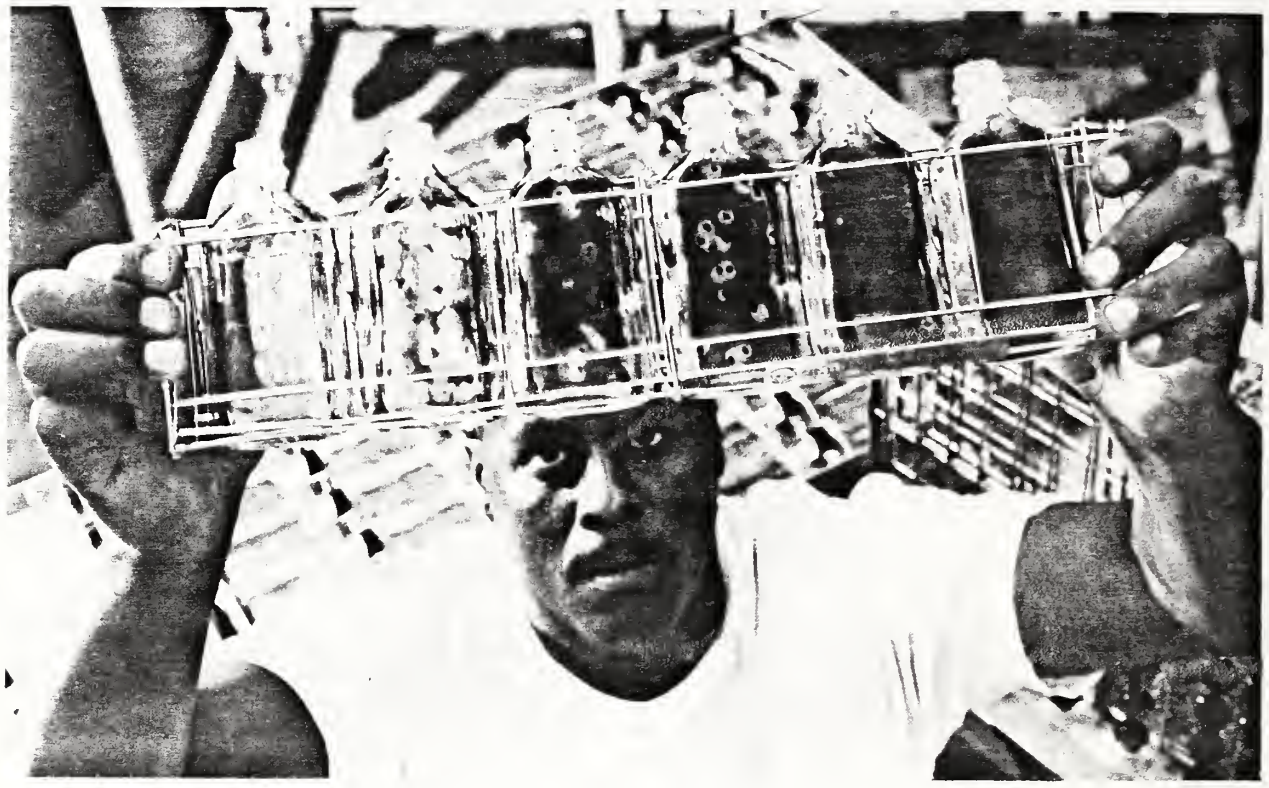
Demonstrated that VIA made from extracts of FMDV-infected cell cultures reacted with antibodies in serums from FMD-infected animals in agar gel precipitin tests, forming a band distinct from that of whole FMDV. A similar finding was demonstrated by means of fluorescent antibody techniques.

Established that the FMDV infection associated antigen used in the VIA agar gel diffusion test was a valuable tool for epizootiological surveys.

Found that FMDV persisted in cell cultures made from the pharyngeal and esophageal cells of infected cattle for as long as 24 weeks.

Found that FMDV can infect cattle, sheep, and goats and multiply in the upper respiratory tract regardless of their immune status, and that this infection and multiplication can occur in the complete absence of clinical signs.

Showed that a steer after intranasal inoculation with FMDV could transmit FMDV for 7 to



PN-3663

A technician shows plaques formed by virus particles found in the blood of steers.

8 days and that the most infectious period was during the third day.

Found a latent form of FMD characterized by virus isolation from the blood in the absence of specific antibody development and with extremely long incubation periods.

Determined that FMDV may be present in semen of infected bulls before and after clinical signs of disease and that it may be transmitted to cows by artificial insemination.

Established that FMDV survives in lymph nodes and blood of beef carcasses for as long as 60 days, in bone marrow for more than 6 months, and in lymph nodes of wet, salt-cured meat for as long as 50 days.

Developed radial immunodiffusion procedures for measuring FMDV in crude tissue culture fluids as well as in concentrated and purified preparations.

Showed that FMDV could be spread by air from infected to clean areas.

Established a facility for the large-scale cultivation of baby hamster kidney cells in rolling bottles and the production, therefrom, of 100 milligrams per week of purified FMDV.

Determined the dose of ionizing radiation required to inactivate FMDV and its RNA.

Found that FMDV is inactivated by organic acids, and by ethylene oxide gas, when sufficient humidity is present, and that beta-propiolactone, acetylethyleneimine, ethylene oxide may be used as inactivants when retention of antigenicity is desired.

Used polyethylene glycol for precipitation of FMDV for vaccine production.

Showed that a FMDV vaccine combining oil adjuvant can be used in a vaccination program involving revaccination, reducing the number of vaccinations per year and giving adequate protection for 6 months or longer.

Determined that purified FMD viruses inactivated and combined with oil adjuvants produced the first satisfactory vaccine for swine.

Showed the possible relationship between swine vesicular disease virus and Coxsackie B_5 virus of humans.

PN-3664

Plaques formed by foot-and-mouth disease virus particles in tissue culture cells.

Other Diseases

Isolated vesicular stomatitis virus from an infected human being.

Developed a rapid laboratory diagnostic test for African swine fever, in cooperation with the East African Veterinary Research Organization.

In cooporation with the same group, established methods for growing the schizont form of East Coast fever parasites in cell cultures. This technique modifies the parasite for potential use in a vaccine.

Developed, in cooperation with the East African Veterinary Research Organization, diagnostic tests for contagious bovine pleuropneumonia in formalized lung tissues using fluorescent antibody and agar gel diffusion techniques.

Purified the attenuated duck enteritis virus and developed a seed virus, which was supplied to the duck industry for production and use as a vaccine. This work was made possible by cooperation of Dutch scientists who supplied the starting materials.

THE CENTER'S FUTURE

As the world human population increases and the food supply becomes less abundant for each individual, the need to reduce losses from animal diseases becomes more important. The Center already has found and will continue to find new ways such as rapid diagnostic tests, control measures, and vaccines to limit or prevent outbreaks of foreign animal diseases.

Increased demand for food supplies involves developing faster growing and improved types of livestock, which in turn requires importation of animals and semen with the special genetic background to develop inbred and hybrid progeny of the desired type. Here again the Center is called upon to develop sensitive tests for detecting disease agents so that such importations may be made with a minimum of risk. Thus the Center has an important role in the development of future food supplies from livestock.

Basic research at the Center should continue to develop new techniques and concepts. However, applied research will receive more emphasis than it has in the past to put into service the improved techniques and findings that have been made through basic studies. Also, the results will have application to many other branches of medical science.

The very nature of research prevents the prediction of the exact character and the timing of conclusive results, but achievements of the program already have been outstanding. In the years ahead, the Plum Island Animal Disease Center undoubtedly will continue to add to the achievements of U.S. and international research.

Prepared by

The Plum Island Animal Disease Center,
Northeastern Region, Agricultural Research Service

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHEASTERN REGION
PLUM ISLAND ANIMAL DISEASE CENTER
POST OFFICE BOX 848
GREENPORT, LONG ISLAND, NEW YORK 11944

April 1978

Infectious Animal Diseases currently being worked with at PIADC or in storage

The first six (6) diseases listed comprise those that account for 90% of the research effort at PIADC as of this date. The bibliography for these diseases listed as having caused laboratory infections is available in the Safety Office. Any questions regarding this list or laboratory infections should be addressed to either the Director or Biological Safety Officer, PIADC.

DISEASE	LABORATORY INFECTIONS	
	Recorded in the literature	PIADC
1. Foot-and-Mouth Disease	Yes	Yes*
2. African Swine Fever	No	No
3. Influenza Type A Swine Equine Fowl	Yes	No
4. Swine Vesicular Disease	Yes	No
5. Rinderpest	No	
6. Sheep Pox	No	
African Horse Sickness	No	
Akabane	No	No
Bluetongue	No	No
Borna	No	
Bovine Enterovirus	No	No

* 1 case, inapparent infection following accidental needle inoculation.

DISEASE	LABORATORY INFECTIONS Recorded in the literature	PIADC
Bovine Mammillitis	No	
Contagious Bovine Pleuropneumonia	No	
Contagious Caprine Pleuropneumonia	No	
Contagious Ecthyma	No	
Contagious Agalactia	No	
Coxsackie B-5	Yes	No
Duck Plague	No	
East Coast Fever	No	
Ephemeral Fever	No	
Epizootic Hemmorrhagic Disease	No	No
Goat Pox	Yes	No
Hog Cholera	No	
Ibaraki	No	No
Infectious Bovine Rhinotraechitis	No	No
Louping Ill	Yes	No
Lumpy Skin Disease	No	
Malignant Catarrhal Fever	No	No
Nairobi Sheep Disease	No	
Newcastle Disease	Yes	Yes
Rida/Visna Disease	No	
Rift Valley Fever	Yes	No Work
San Miguel Sealion Virus	No	

DISEASE	LABORATORY INFECTIONS Recorded in the literature	PIADC
Scrapie	No	
Sweating Sickness of Cattle	No	
Teschen Disease	No	
Venezuelan Equine Encephalitis	Yes	No
Vesicular Exanthema of Swine	No	
Vesicular Stomatitis Virus	Yes	Yes
Wesselsbron	Yes	No Work
T. Parva	No	No
WEE (Western Equine Encephalitis)	Yes	No Work
Trypanosoma Brucei	No	No
Trypanosoma Congolense	No	No
Causative organism of CEM (a bacterium)	No	No
Proteus Mirabilis	No	No
Klebsiella species	No	No
Bacteriophage R-17	No	No
Rhinoviruses	Yes	No
Goose Enteritis Virus	No	No
Hemophilus Pleuropneumoniae	No	No
Eastern Equine Encephalitis	Yes	No Work
Bovine Rotavirus (UK strain)	No	No
Encephalomycarditis viral (EMC) RNA	No	No
Ovine Progressive Pneumonia	No	No

APPENDIX III

EXOTIC PLANT PATHOGENS CURRENTLY UNDER INVESTIGATION AT THE PDRL

Pathogen	Plant Disease
Sclerospora sorghi *Sclerospora philippinensis* *Sclerospora sacchari*	Downy mildew of corn and sorghum
Phakopsora pachyrhizi	Soybean rust
Puccinia polysora ("southern rust") *Puccinia sorghi* ("common rust") *Physopella zeae* ("tropical rust")	Corn and sorghum rust

APPENDIX IV

SELECTED EXAMPLES OF MATERIALS REMOVED FROM PLUM ISLAND LABORATORIES, DECONTAMINATED BY ETO STERILIZATION (1977)

Item(s)	Approximate Value
For Return to Vendor, Manufacturer, etc*	
electronic control boards, vaccine plant	$ 350
flow cells for spectrophotometer	200
circuit boards for electron microscope	300
parts for Multiple Automated Sampling Harvestor	200
pH Electrode	90
analytical balance	300
centrifuge rotor	500
microscope body, lenses, power supply	2300
parts for amino acid analyzer	500
fraction collector	900
vacuum pump	150
calculators	1500
camera equipment	500
movie film	800
Transfer to Other Laboratory on Island	
microscope	15,000

*defective parts, wrong items shipped, repair or modification

APPENDIX V

SELECTED EXAMPLES OF MATERIALS REMOVED FROM PLUM ISLAND (NON-LABORATORY), DECONTAMINATED BY ETO STERILIZATION (1977)

Items	Approximate Value
various calculators	$1500
electronic circuit boards	200
telecopier, radios, tv monitors, communications equipment	1500
motors for rewinding	100-500 each
computer terminal	900
photographic equipment (cameras, projectors)	2300
xerox copier parts	200
film for off-island processing	1200
automotive parts	300